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COMPARATIVE STUDIES OF GROWTH IN DECIDUOUS AND EVERGREEN TREES

R. F. DAUBENMIRE AND M. E. DETERS

Introduction

Several decades ago the School of Forestry of the University of Idaho established an arboretum on the campus at Moscow, Idaho. Although located in a prairie region, the majority of the exotic trees planted there have survived and grown well. Possibly this resulted in part from the fact that advantage was taken of the shelter provided by contiguous north-facing and east-facing slopes, but the trees were planted in closed stands and are not irrigated. Thus many species of trees with wide areas in the North Temperate Zone are growing together under a summer-dry climate to which none is native but to which all have become adjusted. The writers looked upon this as an excellent opportunity to make certain comparative studies of growth and related phenomena.

Methods

A new type of precision dendrometer described elsewhere (1) was used to measure radial changes. In all instances the instruments were located on the north sides of the trunks, between 1.0 and 1.5 meters above the ground. Two or three thrifty trees of each species or variety were measured at intervals throughout the calendar years of 1944 and 1945. In general, radial changes of different specimens of the same species corresponded so closely that none of the conclusions reached would be changed if based on the record from only one tree.

The use of more than one tree was of most value in enabling interpolations when a single record was lost because of vandalism or other causes.

During the early part of the growing season measurements were made on alternate days, but during less critical periods at intervals of a week or more. On rainy days in any season there are practically no daily reversible changes in radii so that significant measurements can be made at any time of day, but at other times useful observations could be made only under special conditions. During the warm season and especially during the period in which active growth began, measurements were made as early after dawn as possible so that the data would not be affected by temporary diurnal shrinkage resulting from vigorous transpiration. During the cold season only those measurements were used which were made in the morning when air temperature was at approximately freezing or above, in order to minimize the influence of thermal shrinkage which begins to take place when the temperature falls below the freezing-point of water.

Since the aim has been to make qualitative comparisons of radial changes, the latter have been expressed in graphs as percentages of the total increment for each calendar year. This method has the advantage of reducing to a minimum the effects of age, vigor, and competition but has a disadvantage in that the degree of

radial fluctuations in trees which show little total growth is exaggerated in comparison with others. Since the net growth (table 1) has been taken into account in interpreting the graphs, it is thought that the advantages of the percentage method far outweigh this single disadvantage.

TABLE 1
SPECIES AND VARIETIES OF TREES STUDIED AND
NET ANNUAL INCREASES OBSERVED IN RADII

TREES	NET ANNUAL INCREMENT (MM.)	
	1944	1945
<i>Evergreen conifers:</i>		
<i>Abies concolor</i> (Gord. & Glend.)		
Hoopes.....	3.28	3.08
<i>Picea engelmannii</i> Parry.....	2.31	1.98
<i>P. pungens</i> Engelm.....	1.42	1.59
<i>Pinus monticola</i> Dougl.....	1.73	1.97
<i>P. ponderosa</i> Laws.....	3.78	5.74
<i>P. strobus</i> L.....	2.26	1.49
<i>Pseudotsuga taxifolia</i> (Poir.) Britt.	2.63	2.06
<i>P. taxifolia</i> var. <i>glauca</i> (Mayr)		
Sudw.....	2.26	1.78
<i>Deciduous conifers:</i>		
<i>Larix occidentalis</i> Nutt.....	3.82	3.11
<i>Deciduous dicotyledons:</i>		
<i>Acer pseudoplatanus</i> L.....	5.26	4.16
<i>A. saccharophorum</i> K. Koch.....	3.01	2.77
<i>Fagus grandifolia</i> Ehrh.....	4.93	3.86
<i>Fraxinus americana</i> L.....	2.42	2.24
<i>Juglans nigra</i> L.....	1.33	1.47
<i>Quercus borealis</i> Michx. f.....	5.16	3.90
<i>Robinia pseudoacacia</i> L.....	1.47	0.86
<i>Ulmus americana</i> L.....	1.28	1.31

ENVIRONMENTAL RECORDS

The instruments of a co-operative station of the United States Weather Bureau are located on the campus on a north-facing slope which is quite comparable with the habitat on which the trees are growing. Daily maximum and minimum air temperatures, daily precipitations in excess of 2.54 mm., and openpan evaporation records have been extracted from the data collected at this

station and are presented in figure 1. Temperature and evaporation records have been plotted for only alternate days through the periods for which there are data. Such simplification makes the graphs more readily comprehensible, yet it brings out the magnitude of diurnal as well as of seasonal fluctuations which are ecologically very important. Also included in figure 1 are soil temperature records taken at a depth of 20 cm. on those dates when tree radii were measured. The soil data are based on the average of one measurement on the north-facing and one on the east-facing slope in the arboretum. Because the trees are growing in a closed stand, soil temperature at any one time is quite uniform, and differences between the two slopes are always very slight. The cyclic daily fluctuation in soil temperature at this depth is very small and influences the data but little, since most measurements were made at approximately the same time of day.

Results and discussion

COMPARISONS BETWEEN DECIDUOUS DICOTS AND EVERGREEN CONIFERS

The following discussion is based entirely upon the eight deciduous dicotyledons and the eight evergreen conifers, for which groups average cumulative growth is presented in figure 2. All reference to the deciduous conifer *Larix occidentalis* is deferred to the subsequent discussion. The averages represent median points within the two groups, respectively.

During the 5-month period from December through April, evergreen conifers showed more response to weather changes than did the deciduous dicots. The radii of the latter varied but little from the midwinter zero point and changed less abruptly than those of the

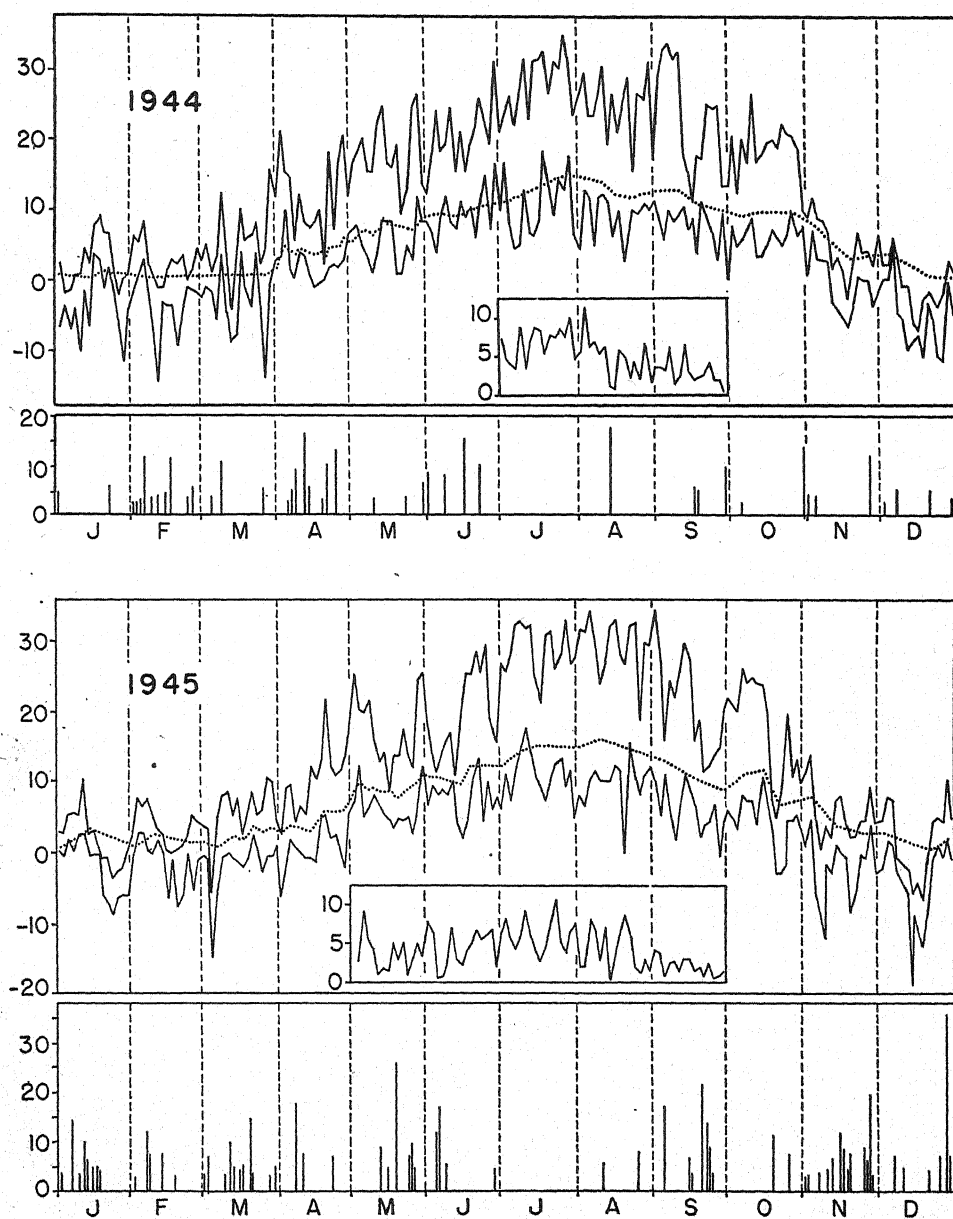


FIG. 1.—Environmental records for 1944 and 1945. For each year: *upper*, maximal and minimal air temperatures, and soil temperature (dotted line) at 20 cm., in °C.; *inset*, open-pan evaporation in mm.; *lower*, precipitation in mm., omitting amounts less than 2.54 mm.

conifers. This difference may be accounted for by the persistent leaves of the conifers, which render these trees very susceptible to shrinkage resulting from vigorous transpiration, and by their softer wood, which may be more responsive to turgor pressure changes.

The data for both years reveal a remarkable identity in the date of beginning of the grand period of growth for the two groups, even though they are unrelated ecologically as well as taxonomically. Both groups grew for a few days in early April, 1944, in response to a brief

continued to show net increases until midwinter. The curves from midsummer to midwinter are very similar both years for the deciduous trees, but the conifers responded quite differently to the differences in weather in the two seasons. The summer of 1944 was not so dry as that of 1945, but the season of inadequate precipitation lasted through October. In 1945 the rains stopped earlier, but the dry season was broken by a period of rainy weather in early September. Thus, the principal rainless season came earlier in 1945.

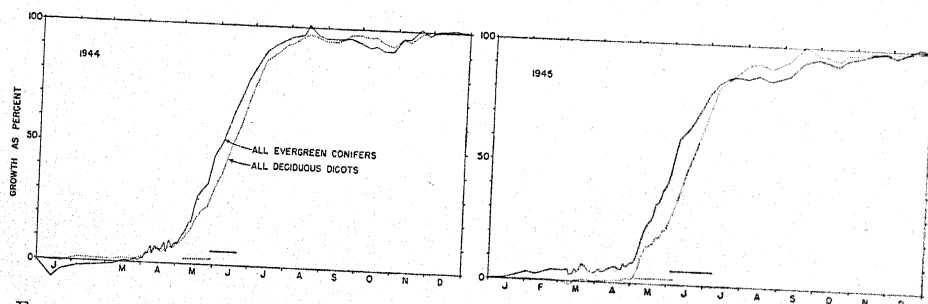


FIG. 2.—Radial changes in stems in percentage of total yearly increment, based on averages of eight species of each group. Horizontal lines explained in text.

period of above-average temperatures, and then remained inactive during a fortnight of cool weather.

Except for about a week in May, 1945, there was a distinct tendency for the evergreen trees to grow more rapidly than the deciduous trees during the early part of the grand period of growth, an advantage which they maintained throughout the summer, even though the rate of increase dropped off earlier in the evergreen trees. In general, the growth rates of the two groups were essentially identical during the period of most active enlargement. During July the rate of growth declined rapidly, especially in the evergreens.

Subsequent to the grand period of growth, radii fluctuated considerably but

The evergreens, which as a group shrank more than the deciduous trees during summer drought, showed their principal response late in the summer of 1944, when the dry season was late, and in 1945 they suffered the most shrinkage earlier in harmony with the earlier occurrence of the dry season.

In the first few months of 1945, a period which in this latitude is generally conceded to be characterized by dormancy, more than 5% of the total annual increment in the evergreens was laid down. This cannot be interpreted as rehydration of tissues that shrank during the preceding summer, for the 1944 record shows clearly that recovery had been complete by the end of the calendar year. It is possible, however, and indeed most

likely, that the January-through-March swelling of the evergreens in 1945 represented belated hydration of tissues formed the preceding summer.

COMPARISONS OF *LARIX* WITH DECIDUOUS DICOTS AND EVERGREEN CONIFERS

The relation of *Larix occidentalis* to the other trees is unique, since it is a conifer and yet has the deciduous habit of the dicots. Although the study of this species was not begun until March, 1944 (fig. 3), this date was well in advance of the time when any tree showed irreversible increases in radius.

In late winter *Larix* exhibited a degree of radial stability that resembled the deciduous dicots more than the evergreen conifers. This supports the hypothesis expressed above that the radial instability which characterizes the latter group is related to their retention of needles and is therefore directly correlated with transpiration.

The grand period of growth of *Larix* began slightly later than the average of each of the two groups. Also, in both summers the shrinkage correlated with drought was more pronounced than that of the average deciduous dicot or evergreen conifer. Thus, the behavior of *Larix* did not consistently resemble that of either deciduous or evergreen trees, except for the matter of radial stability in which it was more like the deciduous dicots.

COMPARISONS AMONG TREES NATIVE TO DIFFERENT ALTITUDES

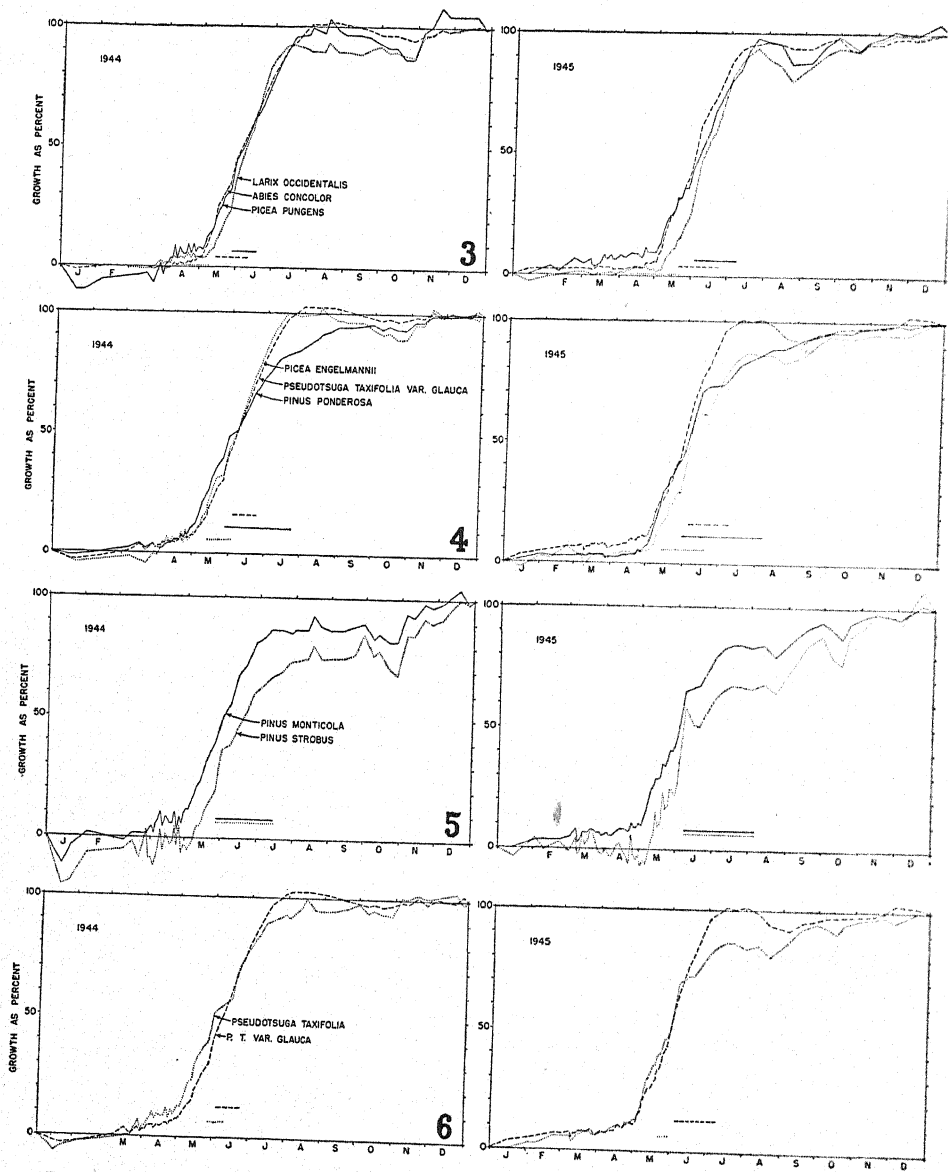
The inclusion of *Pinus ponderosa*, *Pseudotsuga taxifolia* var. *glauca*, and *Picea engelmannii* in the series of conifers under study made it possible to observe any fundamental adaptations to different climates which may exist among species which grow at different altitudes on the

same mountain slope. *Pinus* is native to low altitudes in the Rocky Mountains where the climate is warm and dry. *Picea* occurs at high altitudes where the climate is cool and wet, whereas *Pseudotsuga* occurs at intermediate elevations, overlapping the upper limits of *Pinus* and the lower limits of *Picea*. Obviously, *Pinus* was growing under environmental conditions in the arboretum which most closely approximated its native surroundings, while *Picea* was the farthest from its ecologic optimum.

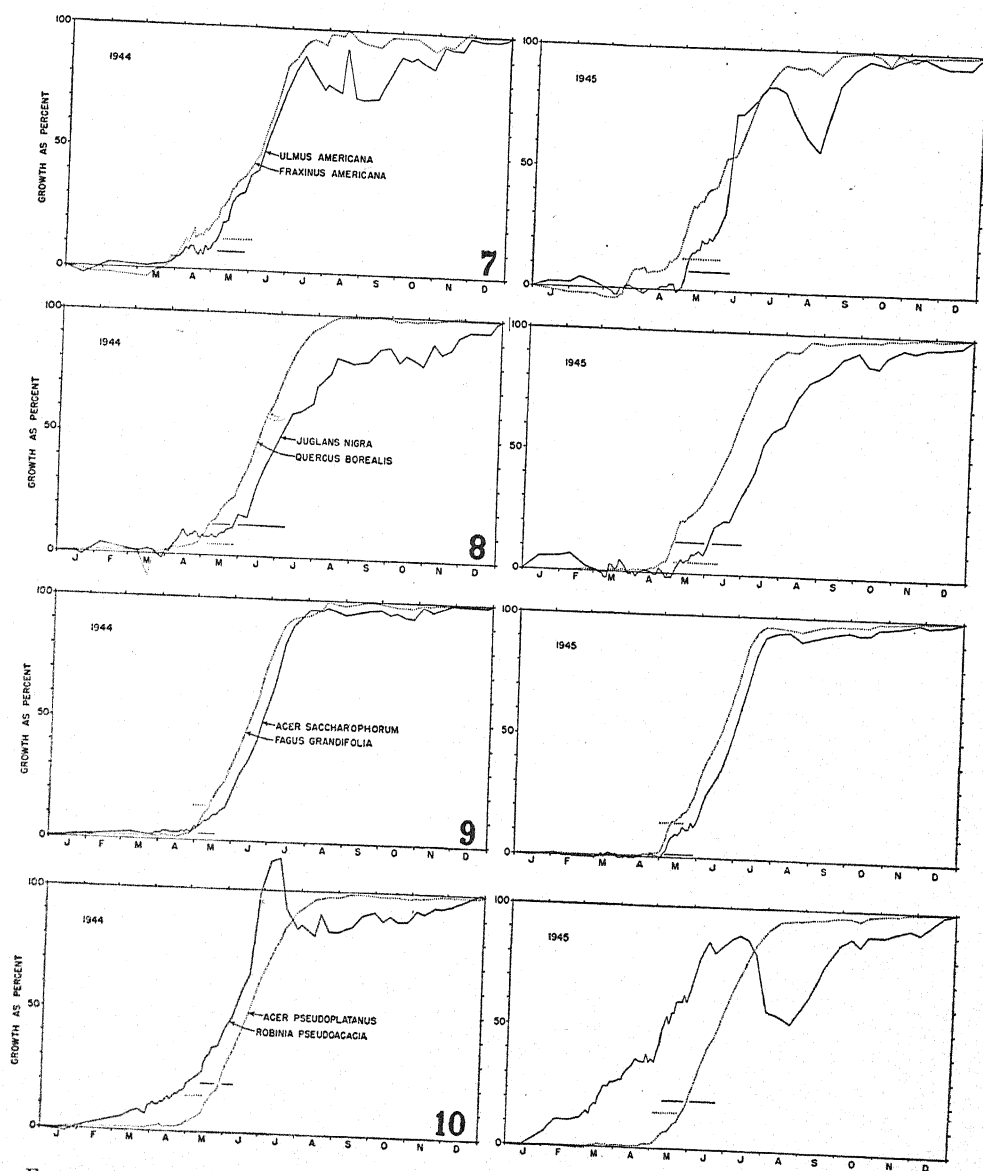
The trunks of *Picea* (fig. 4) were more affected by weather changes from January through April than were those of the other two species, showing pronounced periods of shrinkage and expansion. The radii of *Pinus* fluctuated the least at this season, with *Pseudotsuga* intermediate. Thus the degree of response to variations in winter weather at Moscow was directly correlated with altitudinal distribution.

In 1945 the grand period of growth began slightly earliest in *Pinus* and latest in *Picea*, with *Pseudotsuga* intermediate. This is exactly the reverse of the order which might be expected, assuming that species of cold climates have lower cardinal temperatures than those of warmer regions. However, the data of 1944 show that there is no consistent difference in time of commencement of growth, nor in behavior during the main growing season.

In early June the behavior of *Pinus* diverged considerably from that of the others. The growth rate of this tree was strongly depressed at this time, although rather steady enlargement at an ever slower rate characterized the remainder of the calendar year. *Picea* and *Pseudotsuga*, in contrast, continued rapid growth distinctly later in summer, but then these trees shrank more than did the pine. It is



FIGS. 3-6.—Radial changes in stems in percentage of total yearly increment for species as shown



FIGS. 7-10.—Radial changes in stems in percentage of total yearly increment for species as shown

significant to note that the shrinkage in *Pinus* is essentially autogenous, for, having a much deeper root system than either *Picea* or *Pseudotsuga*, it could not have been affected by the progressive downward desiccation of the soil until after the others had suffered considerably. If the curve for *Pinus* were smoothed, there would be no absolutely quiescent period at any season. This tree at Moscow grows rapidly during May and June, slowly during July, and then continues enlargement at a very slow and somewhat irregular rate throughout autumn and winter until the next grand period of growth begins.

Among other evergreen conifers studied which are native to the Rocky Mountains, *Picea pungens* and *Abies concolor* (fig. 3) have the closest ecologic affinities with *Pseudotsuga*, while *Pinus monticola* (fig. 5) is ecologically the more closely related to *Picea engelmannii*. When the curves of all these trees are compared in relation to altitudinal distribution, it becomes apparent that there are no evident correlations between the native climates and radial behavior at Moscow. The chief possible exception to this may be *Pinus ponderosa* which, in the broader comparison, is outstanding for its early reduction in growth rate and subsequent freedom from shrinkage due to drought.

In the southern Rockies PEARSON (2) has found that apical growth is related to altitudinal distribution in that, whenever two species characteristic of different altitudinal ranges are observed on the same habitat, the one from the higher altitude usually begins growth the earlier. His observations, therefore, indicate successively lower minimal temperatures for apical growth in species from successively higher altitudes. This relationship does not extend to radial growth in the group of trees under study at Moscow.

Nor was there any consistent relationship between altitudinal distribution and the period of leaf growth. Cambial activity in 1945 appeared to begin in exactly the reverse of the order that would be expected from PEARSON's observations on apical growth in the southern Rockies!

COMPARISON OF *PINUS MONTICOLA* AND *P. STROBUS*

The ranges of *Pinus monticola*, native to mountainous regions of western North America, and *P. strobus*, native to eastern North America, do not overlap. However, these species are very closely related ecologically and taxonomically and appear to have been derived from a common Cenozoic ancestor. Both grow in favorably moist soils, but the western species grows under much the more variable climate with respect to diurnal temperature fluctuations. The two groups of trees studied, three individuals in each, are perfectly matched. The stands are adjacent, the individuals are of approximately equal diameter and height, and the plantations were made with equal spacing.

No other trees exhibited as much relative shrinkage in January, 1944, as did these pines (fig. 5), a behavior which may be largely attributable to the extreme softness of their wood. In both 1944 and 1945 *P. monticola* began rapid growth a few days earlier than did *P. strobus*. The only other notable divergences between the species were observed just prior to the grand period of growth when shrinkage and expansions were occasionally antithetic. In general, *P. strobus* appeared to exhibit the more violent responses to weather variations, but this difference is more apparent than real, for its lower net increment, especially in 1945 (table 1), tends to exaggerate the

degree of response. The most conspicuous feature of the curves is the manner in which they parallel each other throughout most of the year.

COMPARISON OF *PSEUDOTSUGA TAXIFOLIA* AND *P. T. VAR. GLAUCA*

The pronounced physiologic differences between *Pseudotsuga taxifolia* from the oceanic climatic region along the Pacific Coast and *P. t. var. glauca* from the more continental climate of the Rocky Mountain region have long been recognized in silviculture, although taxonomists usually have not considered the morphologic differences sufficient to warrant nomenclatorial recognition. The species along the coast grows chiefly on habitats where drought is slight and transitory, while the inland variety is adapted to a climate that is distinctly drier.

Radial behavior in the species and its variety (fig. 6) differed considerably, but for the most part inconsistently, during the 2 years of study. The chief difference observed both years was that the trees from the coast ceased rapid growth earlier than those from the interior; then subsequently the latter showed a more definite response to cumulative summer drought. In this respect the coastal trees bore approximately the same relation to the interior variety that was noted when *Pinus ponderosa* was compared with the latter. Thus the coastal trees, which, on account of the higher rainfall of their native climate, might be expected to exhibit a behavior more characteristic of a mesophyte, gave responses more nearly resembling a species that is distinctly more xerophytic than the interior variety! It is still possible that this difference in behavior may have resulted from ecotypic specialization, for the exact source of the seed of the two plantations

is unknown, and there are a few localities on the Pacific slope that are drier than the wettest areas where *Pseudotsuga* can be found in the interior.

RELATIONSHIP OF WOOD STRUCTURE TO BEGINNING OF RADIAL GROWTH

Several students of tree growth have concluded that trees with ring-porous xylem begin growth earlier than those with diffuse-porous xylem. In the present series of observations the exact time of beginning of radial growth is not easily determined from the radial measurements, but, if it may be assumed that growth among the species began in the same order in which their curves crossed the 10% line, the data obtained at Moscow (table 2) cast considerable doubt as to the fundamental significance of the earlier conclusion.

It is true that some ring-porous species were earlier than some diffuse-porous species, but the dates are almost identical for the ring-porous *Ulmus* (fig. 7) and *Quercus* (fig. 8) and the diffuse-porous *Fagus* (fig. 9). Furthermore, the semi-ring-porous *Juglans* is not intermediate, as would be expected if the hypothesis were significant, but is later than any of the three diffuse-porous species.

When the nonporous conifers are compared with the porous dicots, the 10% point was reached earlier in *Robinia* (fig. 10) and *Fraxinus* (fig. 7), and later in the two species of *Acer* (figs. 9, 10), than the series of dates representing the conifers (table 2).

PERIOD OF FOLIAR DEVELOPMENT

On account of the height and crowded condition of the trees in the arboretum, and because the buds of some trees undergo a long period of gradual swelling, it was not feasible to attempt an accurate

comparison of the beginning of bud activity with that of radial activity. However, records were kept of the period extending from the time when leaves (leaf sheaths of pines) were first visible to their apparent attainment of full size, as viewed from the ground. This period of foliar development is indicated on each graph by horizontal lines of the same types used to designate the seasonal courses of radial change in each species.

RELATION OF TREE GROWTH TO FROST-FREE SEASON

In 1944 a temperature of 0°C . or lower was recorded in the louvered box at the weather station as late as May 3 and as early as September 18. In 1945 the last date was June 14 and the earliest August 20. The frost-free seasons were 138 and 67 days long, respectively.

In 1944 the average tree (fig. 2) exhibited a short period of growth in early

TABLE 2
COMPARISON OF DATES OF ATTAINMENT OF 10% OF NET ANNUAL INCREMENT IN RELATION TO TYPES OF WOOD STRUCTURE

Wood type	Tree	1944	1945	Av.
Ring-porous.....	<i>Robinia pseudoacacia</i>	Mar 29	Feb 6	Mar 4
	<i>Fraxinus americana</i>	Apr 6	Apr 23	Apr 14
	<i>Quercus borealis</i>	May 4	May 3	May 3
	<i>Ulmus americana</i>	Apr 30	May 9	May 4
Semi-ring-porous	<i>Juglans nigra</i>	May 20	May 31	May 25
Diffuse-porous...	<i>Fagus grandifolia</i>	Apr 30	May 7	May 3
	<i>Acer saccharophorum</i>	May 16	May 17	May 16
	<i>A. pseudoplatanus</i>	May 16	May 19	May 17
	<i>Pseudotsuga taxifolia</i>	Apr 30	Apr 23	Apr 27
Nonporous.....	<i>P. t. var. glauca</i>	May 4	Apr 23	Apr 20
	<i>Pinus ponderosa</i>	Apr 28	May 3	Apr 30
	<i>P. monticola</i>	Apr 28	May 2	Apr 30
	<i>Picea engelmannii</i>	May 4	May 7	May 5
	<i>P. pungens</i>	May 6	May 3	May 5
	<i>Abies concolor</i>	May 8	May 5	May 7
	<i>Larix occidentalis</i>	May 14	May 15	May 14
	<i>Pinus strobus</i>	May 16	May 14	May 15

When the dates of leafing seasons are averaged (fig. 2), it appears that the new needles of the evergreen conifers typically began to emerge as the dicot leaves attained full expansion. The needles of the deciduous conifer *Larix* (fig. 3) developed quite early, at a season more characteristic of the deciduous dicots than of the evergreen conifers.

In both 1944 and 1945 radial growth began slightly later in *Pinus strobus* than in *P. monticola* (fig. 5), but in neither year could any difference in the period of foliar development be detected.

April and then began its major growing season immediately after the last frost on May 3. A number of species included in this average began to grow well in advance of the frost-free season. Growth in nearly all trees had ceased many weeks before the first frost in the autumn of that year.

In 1945, 50% or more of the season's growth (fig. 2) was completed before the last frost in spring, and growth had been curtailed by drought well before the first frost in autumn of that year. The data thus show no correlation between the

frost-free season and the season of radial growth. In 1944 the grand period of growth was shorter than the frost-free season; in 1945 it was longer.

About half of the trees were clearly affected by the unseasonal drop in temperature which occurred during the middle of the growing season of 1945, on June 14. Responses varying from a slowing of the growth rate to a pronounced shrinkage that was not compensated for several days or more were observed in the evergreen species *Picea engelmannii*, *Pinus ponderosa*, *P. strobus*, and *Pseudotsuga taxifolia*, and in the deciduous species *Fraxinus americana*, *Juglans nigra*, *Robinia pseudoacacia*, and *Ulmus americana*.

BEHAVIOR OF ROBINIA PSEUDOACACIA

In some respects the radial behavior of *Robinia pseudoacacia* (fig. 10) was unique. In both summers the grand period of growth terminated very abruptly in early July, following which the tree shrank rapidly, losing about 30% of its total annual increment. Shrinkage in the summer of 1944 was not made up until the following February. In 1943 (data not included in figures) shrinkage did not begin until after the first week in August, and recovery was not completed until after the first week in March, 1944.

A second remarkable feature is the earliness of beginning of the grand period of growth. Rapid enlargement began in early March, 1945, whereas the average date for this event in all species was 2 months later (fig. 2)!

Summary

1. Radial changes throughout the years 1944 and 1945 were studied in seventeen species of trees at Moscow,

Idaho, in a region originally covered with prairie. Subsequent to their establishment in an arboretum the trees have not been irrigated, and all have become sufficiently adjusted to the environment to show approximately normal growth.

2. The groups of eight evergreen conifer (*Abies concolor*, *Picea engelmannii*, *P. pungens*, *Pinus monticola*, *P. ponderosa*, *P. strobus*, *Pseudotsuga taxifolia*, and *P. t.* var. *glauca*) and eight deciduous dicots (*Acer pseudoplatanus*, *A. saccharophorum*, *Fagus grandifolia*, *Fraxinus americana*, *Juglans nigra*, *Quercus borealis*, *Robinia pseudoacacia*, and *Ulmus americana*) that were studied began to grow at approximately the same time in both years. During the early part of the grand period of growth the evergreens grew more rapidly than the deciduous dicots, but in the later part of this period the relation was reversed. The evergreens shrank the most in response to drought in summer. Very slow enlargement was observed during autumn and winter in nearly all species, but this was frequently interrupted by periods of shrinkage. In a few instances winter swelling was in part definitely a rehydration of tissues that had suffered marked shrinkage during the preceding summer. In other cases winter swelling probably represented belated hydration of cells produced in summer. The radii of evergreens were less stable during winter than were those of deciduous trees, a phenomenon which is probably related to needle retention, as this habit makes the plants relatively sensitive to transpiration stress in winter.

3. In most respects, radial behavior of the deciduous conifer *Larix occidentalis* did not consistently parallel either that of the other conifers or that of the deciduous dicots, but in its radial stabil-

ity during winter and in its earliness of foliation in spring it resembled the deciduous dicots closely.

4. The inclusion of *Pinus ponderosa*, *Pseudotsuga taxifolia* var. *glauca*, and *Picea engelmannii* in the series of trees provided excellent opportunity to compare the behavior of species native to low, intermediate, and high altitudes in the Rocky Mountains. No consistent differences in behavior were found that could be definitely related to altitudinal distribution. *Pinus ponderosa* exhibited a pronounced autogenous retardation of growth that began early in summer. Subsequently this species did not seem as much affected by the normal summer drought as were the species from higher altitudes, but this behavior appears to be more a peculiarity of the species than a fundamental adaptation to the vertical gradient in climate on mountain slopes.

5. Radial changes in *Pinus monticola* paralleled those of *P. strobus* very closely, except that the former regularly began to grow a few days earlier.

6. *Pseudotsuga taxifolia*, native to the Pacific slope, responded in a manner that indicated a greater degree of xerophytism than possessed by trees representing *P. t.* var. *glauca* of the Rocky Mountain region. The growth of the trees from the coast slowed down earlier in summer, and these trees shrank less in response to late-summer drought. Although there is no record of the exact source of the seed,

this difference in behavior may reflect ecotypic specialization, for the driest habitats supporting *Pseudotsuga* along the coast are drier than the wettest habitats in which the species may be found in the interior.

7. No consistent relationship was found between xylem structure and earliness of cambial activity in spring.

8. In general, the leaves of the deciduous trees were nearly fully developed before the new foliage of the evergreens made its appearance.

9. The growing seasons of the trees began before the frost season had ended, and growth stopped well in advance of the first frost in autumn. A drop in temperature to 0° C. on June 14, 1945, at about the middle of the grand period of growth, temporarily produced either a reduction in the rate of growth or shrinkage in about half of the species of evergreens and in half of the species of deciduous trees.

10. *Robinia pseudoacacia* is unique in exhibiting a marked shrinkage, amounting to about 30% of its net annual increment, which took place abruptly after the grand period of growth had ended in both summers. In 1945 this shrinkage was not made up until the following February or March.

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A COMPARISON OF THE EFFECTIVENESS OF 2,4-DICHLOROPHENOXYACETIC ACID AND FOUR OF ITS SALTS IN INDUCING HISTOLOGICAL RESPONSES IN BEAN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 586

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Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) and its various derivatives have been tested in many experiments and have found wide practical application as plant growth-regulating substances. Among the derivatives, several salts of the acid have been used, especially those which are more soluble in water than the acid itself. In early experiments with 2,4-D and with other substituted phenoxy compounds, ZIMMERMAN and HITCHCOCK (11) and ZIMMERMAN (10) reported that the salts, esters, and amides were approximately equal in activity to the acid. Considerable experimentation since the early work has resulted in a modification of this evaluation. HAMNER *et al.* (3) made the general statement that the esters are more effective than the acid and the acid more effective than the salts. They found that the herbicidal action of the sodium salt could be greatly increased when it was applied in an acid solution. MARTH and DAVIS (6) used the ammonium and the sodium salts as well as the acid in herbicidal spray tests on various weeds. They found all three forms effective in causing the death of a number of plants but suggested that certain plants were more sensitive to the acid than to the sodium salt. ENNIS and BOYD (2) reported after extensive spray treatments of many types of broad-leaved plants that the effectiveness of the ammonium salt was not statistically different from

that of the acid as a growth-regulating substance. On the other hand, TAYLOR (9) found in general that 2,4-D caused greater inhibition of growth than did the ammonium salt when young wheat plants were grown in nutrient solutions containing either of these compounds.

The present experiment compares the growth-regulating activity of 2,4-D and four of its salts on the basis of histological response induced in the bean plant. The treatment was made on young, decapitated plants in a manner similar to that first used by KRAUS *et al.* (5) and later extended to a series of experiments testing various growth-regulating substances, including 2,4-D and related phenoxy compounds recently reported by BEAL (1). Plants of *Phaseolus vulgaris* var. Red Kidney were grown in flats in the greenhouse. After selection for uniformity and vigor of growth the plants were divided into five lots. Each lot received treatment with one of the following compounds mixed in 0.5% concentration by weight in lanolin: 2,4-dichlorophenoxyacetic acid (2,4-D), ammonium 2,4-dichlorophenoxyacetate, copper 2,4-dichlorophenoxyacetate, calcium 2,4-dichlorophenoxyacetate, and magnesium 2,4-dichlorophenoxyacetate. The plants were decapitated in the upper part of the second internode, and the lanolin paste was applied to the cut surface. The decapitated stems were collected at given intervals up to 30 days, killed and fixed in Navashin's fluid, imbedded by the

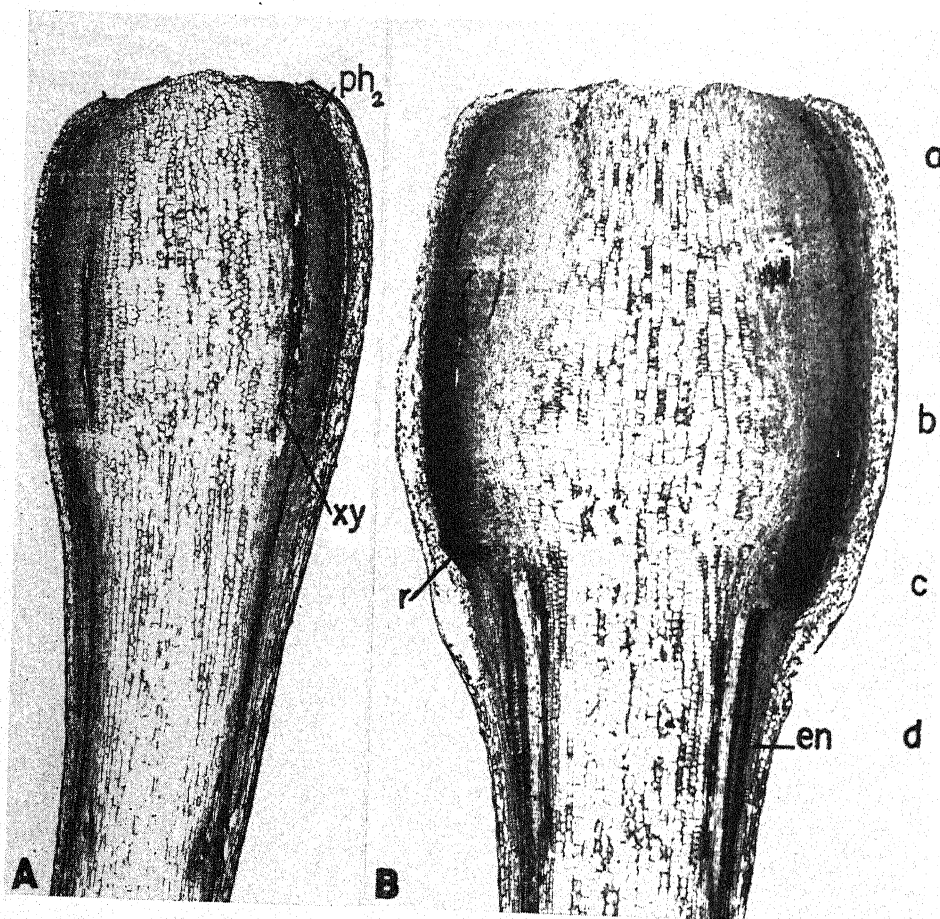


FIG. 1.—Longisections of decapitated second internodes treated with 0.5% 2,4-D. Young stages in enlargement of stem tips to form apical tumors. *A*, 3 days after treatment: *ph*₂, secondary phloem; *xy*, secondary xylem. Parenchymatous tissues from inner cortical parenchyma to pith proliferating. Pith inactive. *B*, 5 days. Further proliferation of parenchymatous tissues and enlargement of tumor; greatest vertical depth of response in endodermis (*en*); initiation of root primordium (*r*). On basis of meristematic activity (generally indicated by density of area) stem tip may be divided into zones: at upper levels, zone of limited proliferation (*a*); at intermediate levels, zone of major proliferation (*b*); at shoulder of tumor, root zone (*c*); at lower levels of response, zone of minor proliferation (*d*).

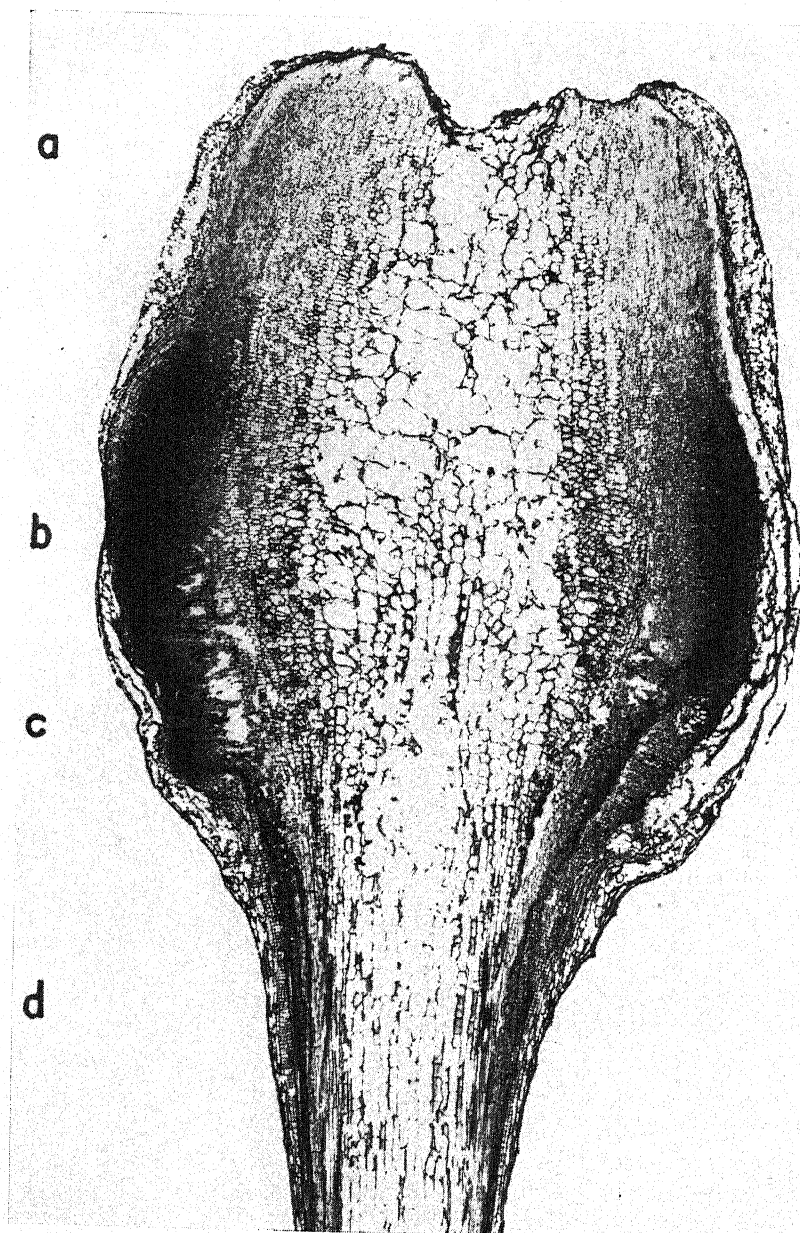


FIG. 2.—Longisection, 12 days after treatment with 2,4-D. Zones *a-d* same as in fig. 1*B*. Mature apical tumor showing zonation in pattern of proliferation and maturation of tissues. Zone *a*, proliferated tissues matured as parenchyma; many dying cells at cut surface, in pith, and from pericycle outward. Zone *b*, derivatives of proliferated tissues from primary phloem inward to pith actively meristematic; outer tissues generally collapsed. Zone *c*, tissues forming root primordia remaining meristematic; other proliferated tissues maturing as parenchyma or as reticulate tracheids. Zone *d*, previously active cells maturing as parenchyma and as scattered reticulate tracheids; occasionally vascular bundles formed, especially in phloem.

butyl alcohol-paraffin method, sectioned at 12-15 μ , and stained with a modified Flemming's triple stain.

Observations

EARLY RESPONSES

Response in decapitated stems was initiated during the first 24 hours. Changes in the appearance of these stem tips, almost imperceptible during the first day, were distinct by the end of the second day. The green color faded, the originally inconspicuous ribbing of the stem became more marked, and the tip began to enlarge in the formation of a tumor. All treatments resulted in a similar series of early reactions. During the third and fourth days the tumors grew considerably in size and began to show characteristic differences. The depth or downward extent of response in the stem was greatest in those tips treated with 2,4-D and with the ammonium salt, somewhat less when the copper salt was used, and distinctly shallower with the calcium and magnesium salts (figs. 1A, 3). The shapes of the tumors likewise varied significantly. The deeper tumors resulting from treatment with 2,4-D and with the ammonium salt were club-shaped and slightly constricted at the top; the tumor induced by the copper salt was intermediate in position, somewhat variable in shape, but often bulbous at the upper end; the shallow tumors induced by the calcium and magnesium salts were flared at the cut surface and gradually tapered below. With all treatments the tumor was greenish-yellow in color, waxy on the lateral surface, and rather dense and firm in texture by the fourth day.

Histological examination very clearly showed the tissue proliferations underlying the changes seen in gross responses.

For all five substances there was fundamental similarity in the tissues which responded and in the type of response induced. Certain variations became increasingly marked, however, with the continued development of the tumors. In the following analysis of early response, development by tissues is traced through the first 4 days after treatment.

The epidermis exhibited no specific response to 2,4-D or to its salts. The outer cortical parenchyma showed little early response aside from cell enlargement. This occurred for several millimeters downward in the stem, extending generally over those portions showing marked increase in diameter. In some cases cell enlargement was followed by cell division. This was a frequent and characteristic response to the magnesium salt (fig. 8A), although it might occur in the cortex over especially active bundles with any of the other substances. Cell division was in various planes, any one cell undergoing only one or two divisions. This response followed the initiation of activity in tissues centrad to it and was not marked until the third or fourth day.

Response in the inner cortical parenchyma was similar to that of the outer cortical parenchyma but was earlier and greater in degree. By the end of 24 hours, cells adjacent to the endodermis showed increase in density of cytoplasm and in size of nuclei. In those stems treated with the magnesium or calcium salt, divisions in various planes occurred during the first day after treatment, and in stems treated with the ammonium salt and 2,4-D during the second day. As proliferation continued, this tissue became indistinguishable from the endodermis.

The endodermis was the first tissue to respond and showed proliferation within approximately 24 hours. Slight cell enlargement (not the radial elongation

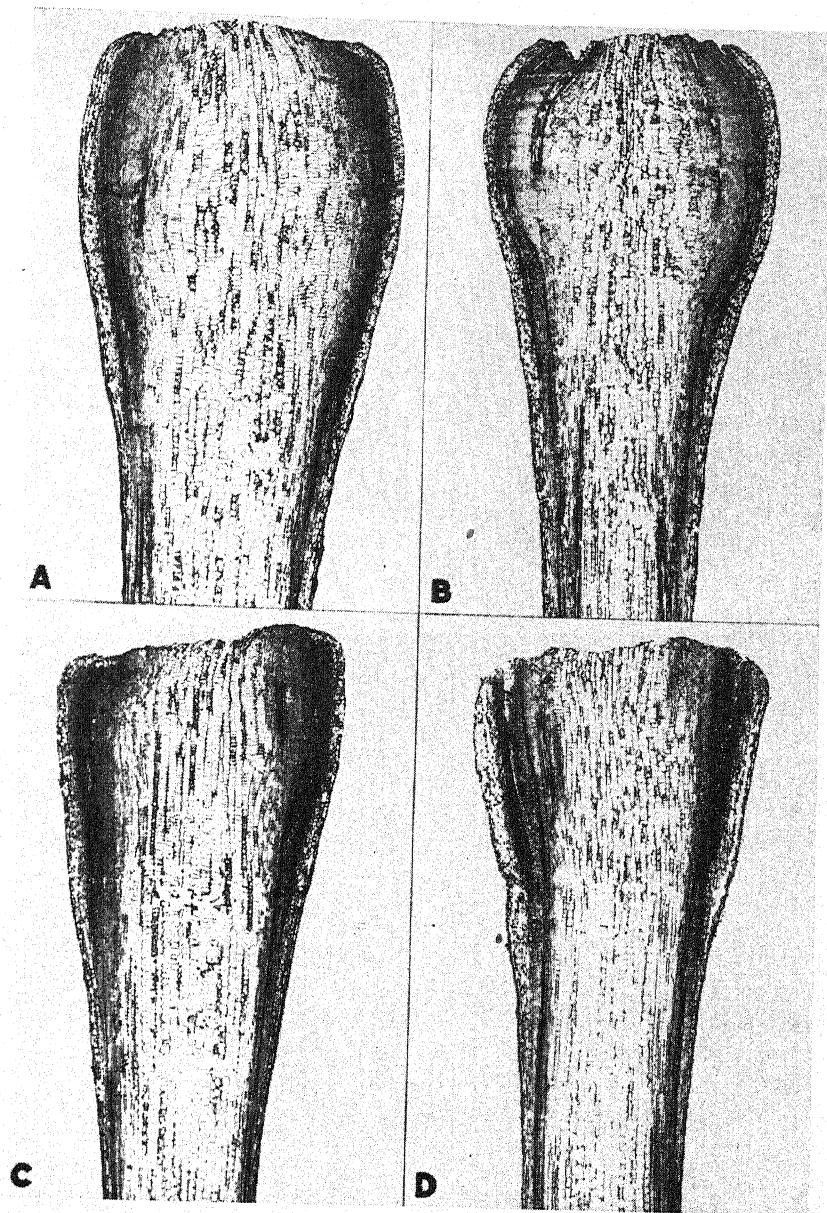


FIG. 3.—Longisections of stem tips 3 days after treatment with 0.5% ammonium (A), copper (B), calcium (C), and magnesium (D) salts of 2,4-dichlorophenoxyacetic acid. Tumors arranged in series from deep, club-shaped tumor induced by ammonium salt to shallow, flared tumor induced by magnesium salt. Initial proliferation of parenchymatous tissues at upper levels well advanced; central pith inactive. Note activity in outer cortical parenchyma of two shallower tumors, C and D. Compare with fig. 1A.

characteristic for some growth substances) and conspicuous increase in density of cytoplasm preceded tangential division. The magnesium salt induced the most marked response during the first day, the calcium salt next, and 2,4-D the least. During the next few days divisions continued, in the radial as well as in the tangential plane. Generally a narrow band of meristematic tissue was produced (fig. 14). Except at lower levels where only the endodermis showed response, this band merged with proliferating tissues on either side of it. The vertical depth of this response at 3 days corroborated the differences among the substances already noted in descriptions of the tumors: 2,4-D and the ammonium salt induced the deepest responses, the copper salt was intermediate in effect, and the calcium and magnesium salts induced only shallow reactions (table 1).

The pericycle is generally less sensitive to treatment with growth-regulating substances than tissues on either side of it. The degree of proliferation described here, and most clearly discernible in longisections, is unusual. At upper levels young parenchymatous pericyclic cells slowly increased in size and the nuclei enlarged, but the cytoplasm did not appear dense. Within 2-3 days divisions occurred in many of these large cells (fig. 14A). The nuclei resulting from these divisions were frequently multinucleolate, a condition observed only occasionally in other tissues. Proliferation of the pericycle sometimes continued to a point where the cells became indistinguishable from other proliferated tissues. However, the pericycle over the vascular bundles generally remained a conspicuous patch of large, thin-walled cells (fig. 8A) which tended to collapse. These cells might collapse as early as 4 days with the copper, calcium, and magnesium salts.

At levels where the pericyclic cells had begun to mature as fibers, response by proliferation did not occur.

Proliferation of the parenchymatous cells of both the primary and the secondary phloem was of major importance in the formation of the tumors. Response in the phloem parenchyma was initiated during the first 24 hours after treatment. The cells gradually became more meristematic in appearance, the cytoplasm denser, and the nuclei larger. Some cells appeared enlarged. Cell division was initiated earliest in those stems treated with the magnesium salt, but by the second day this tissue had begun to proliferate in all stems. Divisions were in all planes, frequently four to five divisions taking place before the outline of the original cell was lost. By the third day at levels of greatest meristematic activity the phloem had formed a wide band of proliferated tissue which merged indistinguishably with adjoining areas. However, the sieve tubes of the secondary phloem remained conspicuously unchanged among the meristematic cells (fig. 14A). At lower levels in the tumor, meristematic activity in the phloem was limited, often not exceeding the first stages where the outline of the original cell was evident. The depth of response of this tissue was next greatest to that of the endodermis and reached within 2-4 mm. of the latter (fig. 14B).

In the cambium, initiation of activity resulting from treatment by the different substances was difficult to determine. At the end of 48 hours the zone of undifferentiated cambial derivatives had widened considerably, and activity had extended across many of the rays. Activity continued through the third and fourth days. In stems treated with the magnesium salt, maturation of xylem tracheids among the inner derivatives of the

cambium commenced by the second day at 0.5 mm. and extended downward. Similar maturation was noted with the other substances by 3 days, at 1 mm. with the calcium salt, at 3 mm. with the copper salt, and at 4 mm. with both 2,4-D and the ammonium salt.

Parenchyma in that portion of the ray lying outside the interfascicular cambium responded in the upper levels in a manner similar to the phloem parenchyma. Together these formed a broad band of small-celled, meristematic tissues. By the fourth day at upper levels in the shal-

outline was lost. The xylem parenchyma attained a similar degree of activity. In many cases inactive lignified primary xylem elements were widely separated by the enlargement or proliferation of intervening parenchymatous cells. Gradually this activity extended inward to the pith parenchyma centrad to the protoxylem points, especially in tumors induced by the magnesium salt. However, the response of this inner portion of the stem was never so great as that in the outer portion. The earliest maturation of proliferated cells occurred among the deriva-

TABLE 1

DEPTH OF RESPONSE IN ENDODERMIS IN TUMORS INDUCED BY 2,4-DICHLORO-PHENOXYACETIC ACID AND FOUR OF ITS SALTS, ON VARIOUS DAYS AFTER TREATMENT

SUBSTANCE	DEPTH OF RESPONSE (MM.)						
	2 days	3 days	4 days	5 days	7 days	12 days	20 days
2,4-D.....	10.3	11.6	15.0	13.5	13.0	12.6	11.5
NH ₄ salt.....	9.5	12.0	14.0	13.0	14.3	13.0	11.0
Cu salt.....	8.0	11.6	14.0	12.6	12.0	11.3	11.6
Ca salt.....	6.5	10.0	11.0	11.0	10.0	10.0	12.3
Mg salt.....	6.0	9.3	9.0	10.0	9.0	9.5	8.3

low tumors and at middle levels in the deeper tumors the meristematic activity of the rays exceeded that of the phloem. This radial pattern of alternating patches of intensely active rays and less active bundles was the first indication of developing root primordia.

The inner ray, that portion extending from the cambial derivatives to the protoxylem points, was markedly less active. During the first day the cells increased in size slightly, and the nuclei became particularly conspicuous. Cell divisions were observed within 24 hours after treatment with the magnesium and calcium salts and within 48 hours with the other substances. Several cell divisions might occur before the original cell

tives of proliferation in the ray and xylem parenchyma. Reticulate tracheids were observed as early as the third day in tumors induced by magnesium and calcium salts, but not until several days later in stems treated with the other substances. The central pith was inactive.

LATER RESPONSES

ZONATION.—By the fifth day the pattern of response in the various tissues was fully established and the structure of the tumor could be studied as a whole. In the early response it was apparent that the degree and character of proliferation varied at different levels. Analysis of these differences disclosed a pattern of zonation. The pattern, as most fully de-

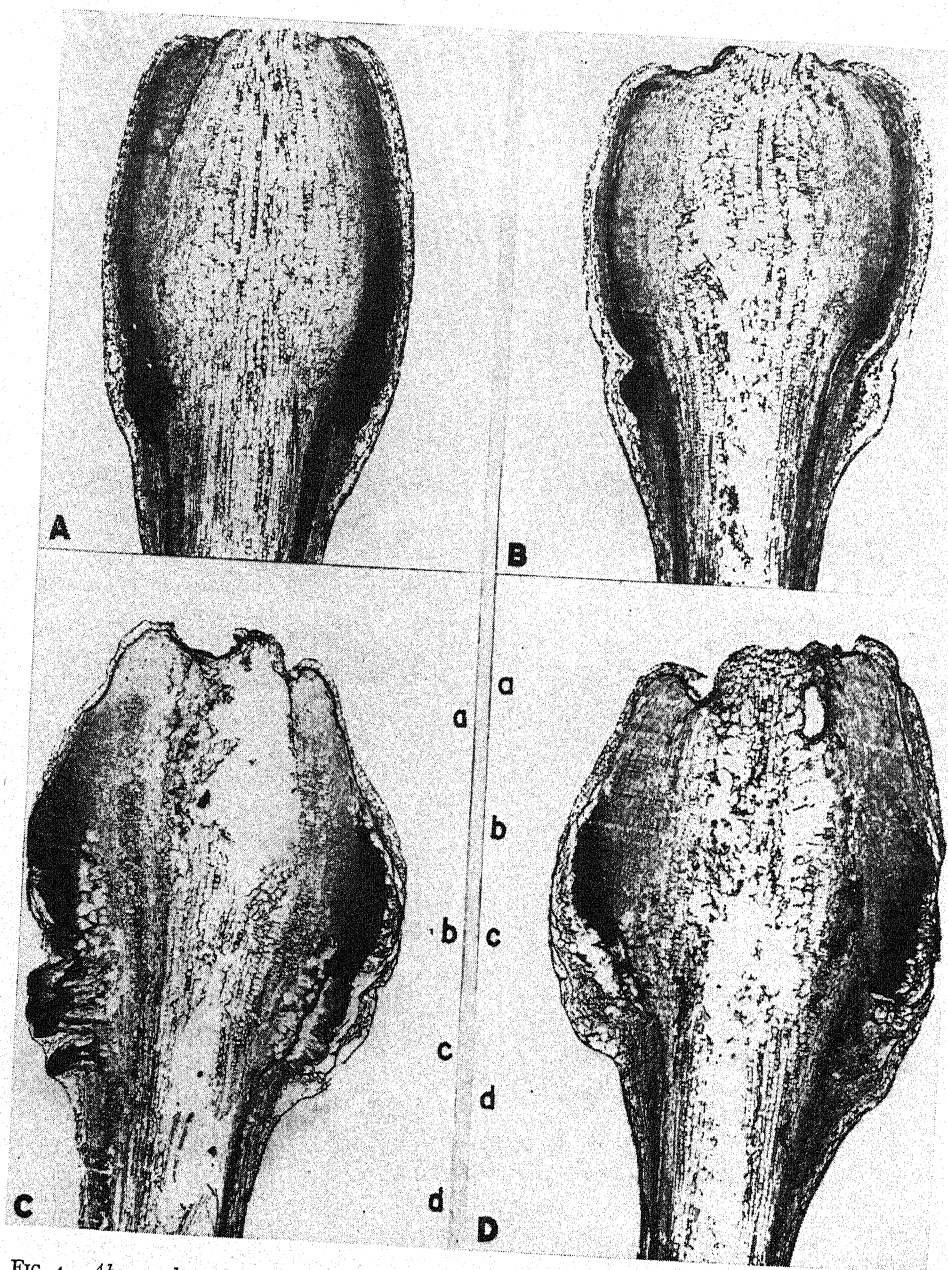


FIG. 4.—Above, 5 days; below, 12 days after treatment. Left, ammonium salt; right, copper salt of 2,4-D. Zones a-d same as in fig. 1B. Zone of limited proliferation evident in deep tumors induced by these salts. In root zone (c) note upper fasciated or multiple root primordium and lower single primordium in C.

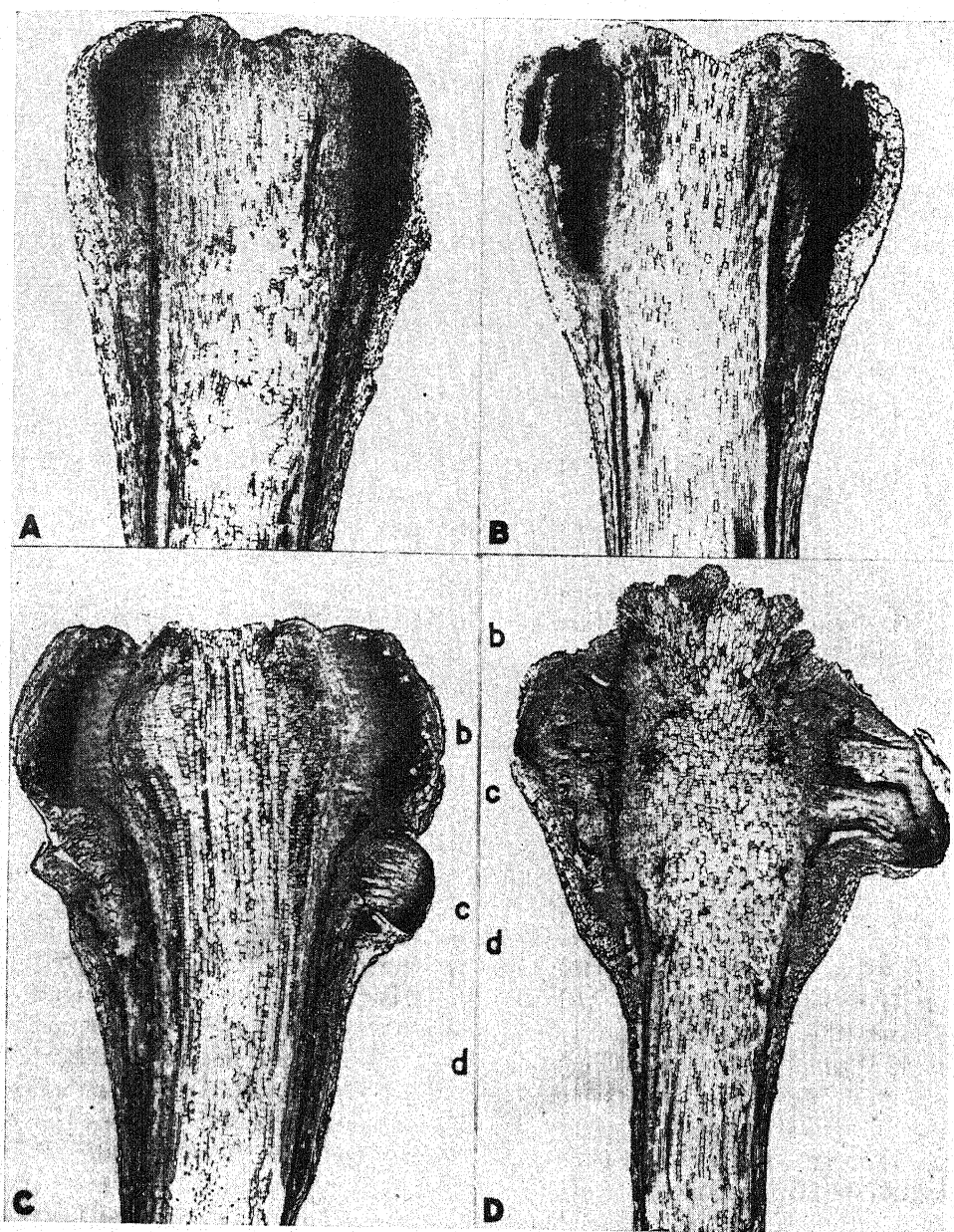


FIG. 5.—*Above*, 5 days; *below*, 12 days after treatment. *Left*, calcium salt; *right*, magnesium salt of 2,4-D. Zones *b-d* same as in fig. 1B. Zone of limited proliferation lacking for shallow tumors induced by these salts. Proliferation above original level of cut surface characteristic response to magnesium salt. Figures 4 and 5 show variation in form of tumors resulting from treatment with different salts of 2,4-D and are arranged to indicate decreases in vertical depth of response with accompanying changes in zonation. (See also table 2). Characteristic levels of root zone (*c*) are shown: lowest with ammonium salt (fig. 4C) and nearly at cut surface with magnesium salt (fig. 5D). Figs. 3, 4, 5 same magnification.

veloped, consisted of four zones. These zones (figs. 1B, 2), arranged in order from the cut surface to the lowest depth of response in the tumor, were as follows: (a) zone of limited proliferation; (b) zone of major proliferation; (c) root zone; and (d) zone of minor proliferation. Each zone was distinctive in its position and in its histological character, although there was a gradual transition between the zones. The presence or absence of a zone and the distance of each zone from the cut surface were consistent factors in differentiating response to one substance from response to another substance.

In the zone of limited proliferation, response was initiated but was soon inhibited in its further development. This zone was found at upper levels just below the cut surface. During the first 3 days after treatment parenchymatous tissues from the cortex to the inner ray and about the xylem points proliferated in a manner already described under early response. During the fourth and fifth days this rate of proliferation appeared to diminish and the meristematic character of the cells gradually lessened (fig. 6A). Derivatives remained small-celled and parenchymatous (fig. 6B). In decapitated stems treated with 2,4-D and with the ammonium and copper salts, the zone of limited proliferation was typically developed and appeared as the constriction at upper levels of the tumor (figs. 1B, 2, 4). In the flared tumors induced by the calcium and magnesium salts this zone was absent (fig. 5).

In the zone of major proliferation the greatest and most prolonged meristematic activity occurred. Derivatives of cortical parenchyma, endodermis, phloem parenchyma, cambium, and the outer portion of the ray possessed the dense cytoplasm and large nuclei of dividing

cells (fig. 7A). Parenchyma in the inner portion of the ray and about the xylem points responded with considerable enlargement and some proliferation (fig. 7B). A broad and continuous band of active tissue was formed around the circumference of the stem. This wide band of meristematic tissues was particularly evident in tumors induced by 2,4-D and by the ammonium and copper salts. In these tumors the zone of major proliferation was found below the zone of limited proliferation (figs. 1B, 2, 4) and was mainly responsible for the enlargement of the decapitated stem.

In the transition to the root zone the tissues of the rays became more and more intensely meristematic. On alternate radii derivatives of the vascular tissues appeared less active. This type of organization was characteristic of response to the calcium and magnesium salts at the uppermost levels (fig. 9B) and represented the greatest extent of proliferation in these flared tumors (fig. 5).

In the root zone the organization of a primordium occurred generally within each ray. The increased intensity of meristematic activity in the outer rays was observed by the fourth day. By the fifth day root histogens were distinguishable in all treatments. In longisection it was evident that some primordia were continuous vertically over a distance of several millimeters and developed in a fasciated manner with no separation into individual, well-organized root tips (figs. 4C, 5D). From other primordia well-developed individual roots were formed (fig. 4C). With all five substances the root zone was unmistakably expressed in protuberances forming an abrupt ridge or shoulder at the base of that portion of the tumor showing the greatest enlargement. Although the distance of the root zone from the surface of application dif-

ferred markedly in the responses induced by 2,4-D and its salts, the pattern of proliferation within the stems varied but little (figs. 10, 11). In the vascular bundles alternating with the root primordia, proliferation was relatively slight and the pattern similar to that in the zone of minor proliferation.

The zone of minor proliferation was found at levels where response progressively diminished and finally terminated. Although the total proliferation for this portion of the stem was relatively slight, activity in large bundles was conspicuous, especially when the proliferation was followed by a complex pattern of maturation (fig. 12). In the upper part of this zone considerable proliferation of the endodermis occurred over the large bundles (fig. 12*B*). In stem tips treated with the magnesium salt this proliferation frequently extended through the cortical parenchyma to the epidermis. Similar activity was observed in tumors induced by the calcium salt (fig. 15) but was uncommon and much less marked with the other substances. In all treatments a narrow band of proliferated endodermis was formed over the smaller bundles (fig. 12). At the lowest levels endodermal response decreased to a single division and finally to slight cell enlargement. Pericyclic cells (fig. 15) were often matured as fibers; sometimes the cells were enlarged and proliferated. The greatest proliferation in this zone was observed in the phloem parenchyma, both primary and secondary. Activity in this tissue was marked to a depth almost as great as that in the endodermis (fig. 14*B*). Only traces of response were evident in the cambial region, in the xylem parenchyma, and in the tissues of the ray. Limitation of proliferation principally to the vascular bundles was characteristic of response in

the zone of minor proliferation. With all treatments, general maturation of proliferated tissues was found earlier in this zone than in the other zones. Many of the characteristics of the mature condition were evident by the fifth day, especially in those tumors resulting from treatment with the calcium and magnesium salts.

MATURATION.—The greatest rate of enlargement of the tumor occurred during the first week after treatment. Growth thereafter was slower but continued for several weeks more. This growth took place mainly in the zone of major proliferation and in the root zone. The time of maturation and death of the tissues varied in the different tumors. In tumors induced by 2,4-D and by the ammonium and copper salts collapse was evident in the zone of limited proliferation 2 weeks after treatment. Constriction at the tip increased greatly, the tissues changed in color from waxy yellow to brown, and necrosis occurred. By 30 days collapse and death had extended to the lowest levels of response. Decapitated stems treated with the calcium and magnesium salts were firm and green 3 weeks after treatment. By 30 days some collapse and dying were evident. Tissues proliferating above the cut surface in those tumors induced by the magnesium salt remained succulent and active to the end of the period of observation. This was the most prolonged stimulation of tissues noted in this experiment.

In the zone of limited proliferation activity was much reduced by the fifth day. In the small-celled derivatives the cytoplasm was less dense and the nuclei diminished in size. Further development seemed to be inhibited, and the tissues remained parenchymatous for some time (fig. 6*B*). At the end of 12 days the beginning of collapse and death was evident. Commencing at the cut surface and

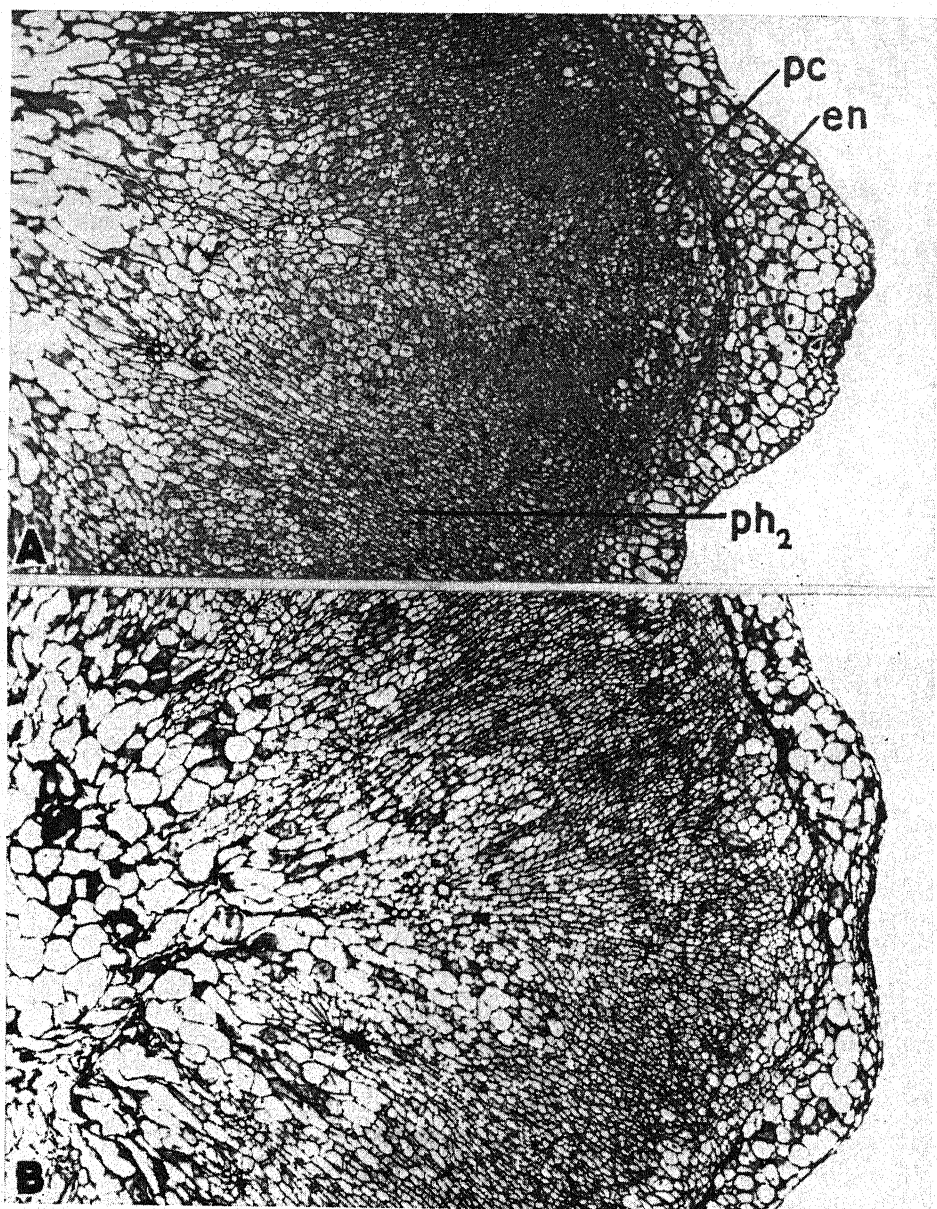


FIG. 6.—Transections of stem tips treated with ammonium salt of 2,4-D; in zone of limited proliferation. *A*, 5 days after treatment, 0.2 mm. below cut surface. Inner cortical parenchyma and endodermis (*en*) slightly proliferated, maturing as parenchyma; pericycle cells (*pc*) much enlarged and some divided; derivatives of proliferated tissues from primary phloem to pith losing meristematic character. *B*, 12 days after treatment, 1 mm. below cut surface. Response limited to degree of proliferation reached by 5 days. Some tissues collapsing; others matured as parenchyma. Compare with fig. 4C.

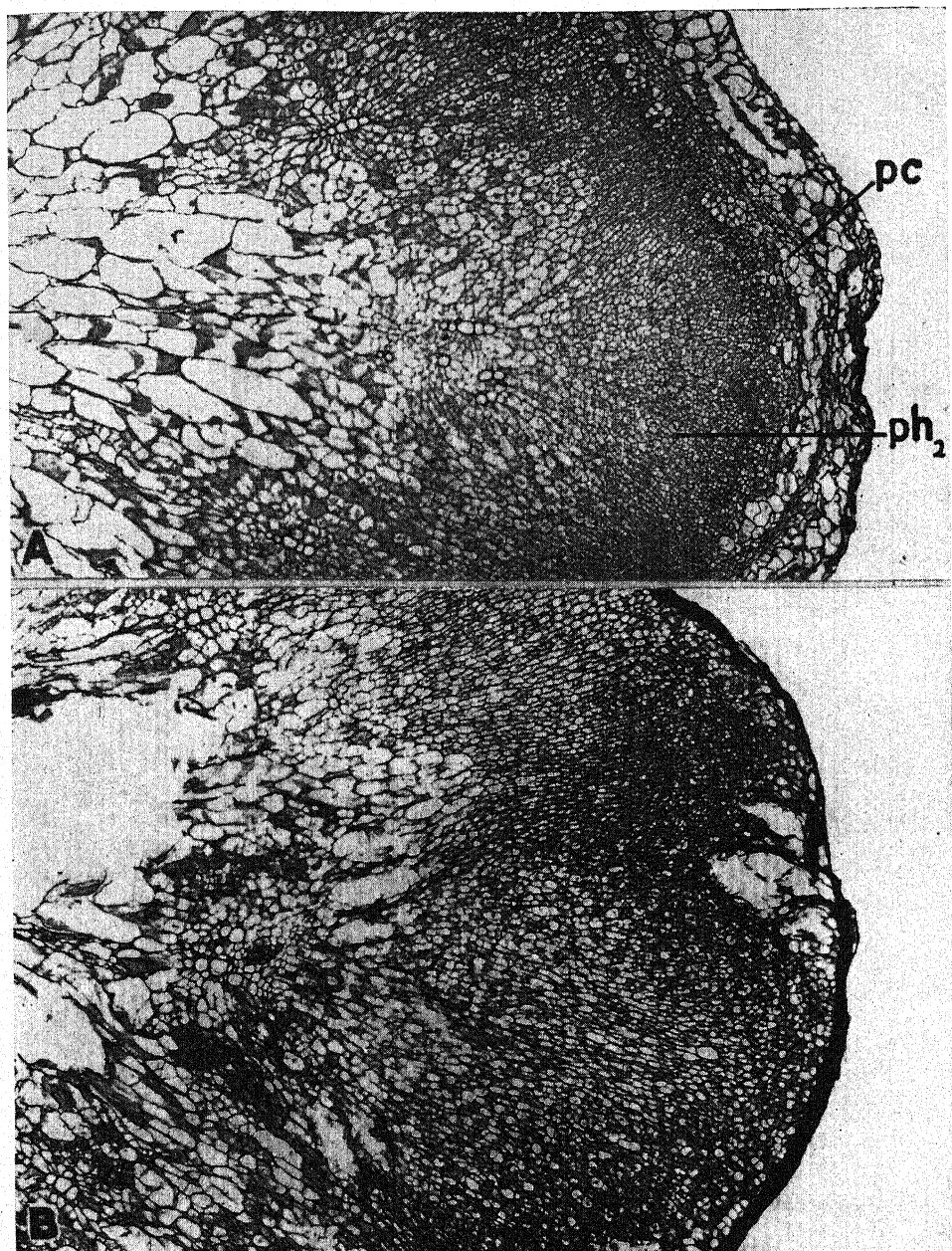


FIG. 7.—Transsections of stem tips treated with ammonium salt of 2,4-D; in zone of major proliferation. *A*, same stem as fig. 6*A*, 3 mm. below cut surface: *ph*₂, secondary phloem. Pericyclic cells much enlarged (*pc*), some collapsed. Parenchymatous tissues from primary phloem to pith strongly meristematic and proliferating; outer portion of rays similarly active. Slight proliferation in xylem parenchyma and in inner portion of rays. Note density of cytoplasm in proliferating cells as compared with fig. 6*A*. *B*, same stem as fig. 6*B*, 4 mm. below cut surface. Collapsed epidermis, cortical parenchyma, endodermis, and pericycle forming heavy outer layer of tumor. Proliferated tissues (phloem, outer portion of ray, and cambial derivatives) generally maturing as parenchyma; some xylem tracheids differentiated. Proliferated cells in xylem parenchyma and in inner portion of ray maturing as reticulate tracheids or remaining meristematic to form vascular bundles.

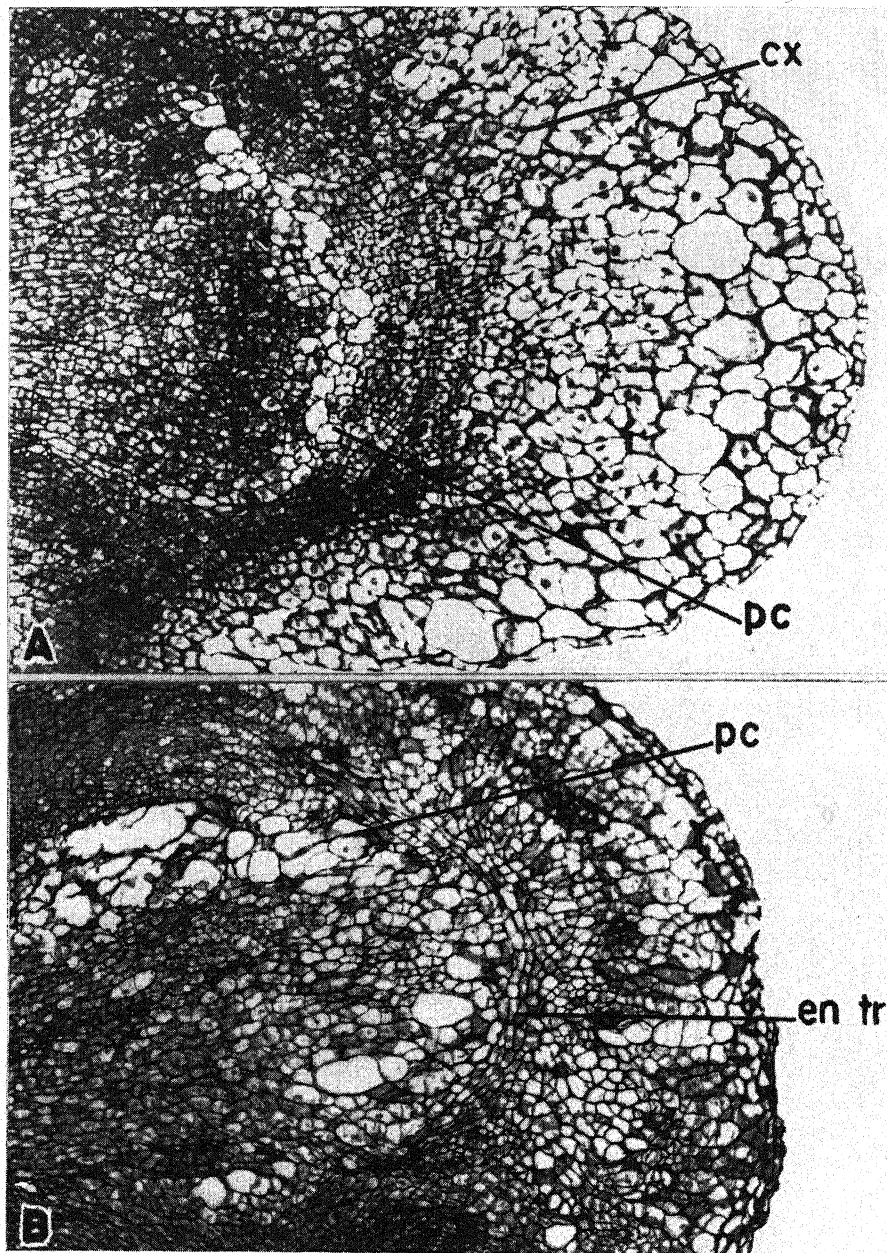


FIG. 8.—Transections of stem tips treated with magnesium salt of 2,4-D. *A*, 4 days after treatment, 1 mm. below cut surface. Greatest proliferation with magnesium salt found at uppermost levels and at this age. Response similar to that noted with ammonium salt at base of zone of major proliferation (5–6 mm.) at 5 days; differences in degree of proliferation between vascular bundles and rays result in scalloped band of active tissues. Note strong proliferation of cortical parenchyma (*cx*) and endodermis, frequent response to magnesium salt. *B*, 5 days after treatment, 0.3 mm. below cut surface. Maturation of proliferated tissues much earlier than that observed in treatment with ammonium salt. (Compare with fig. 7*A*.) Derivatives of proliferated cortical parenchyma, endodermis (*en tr*), and phloem parenchyma maturing as reticulate tracheids or as parenchyma. Some pericyclic cells much enlarged (*pc*), a few collapsed.

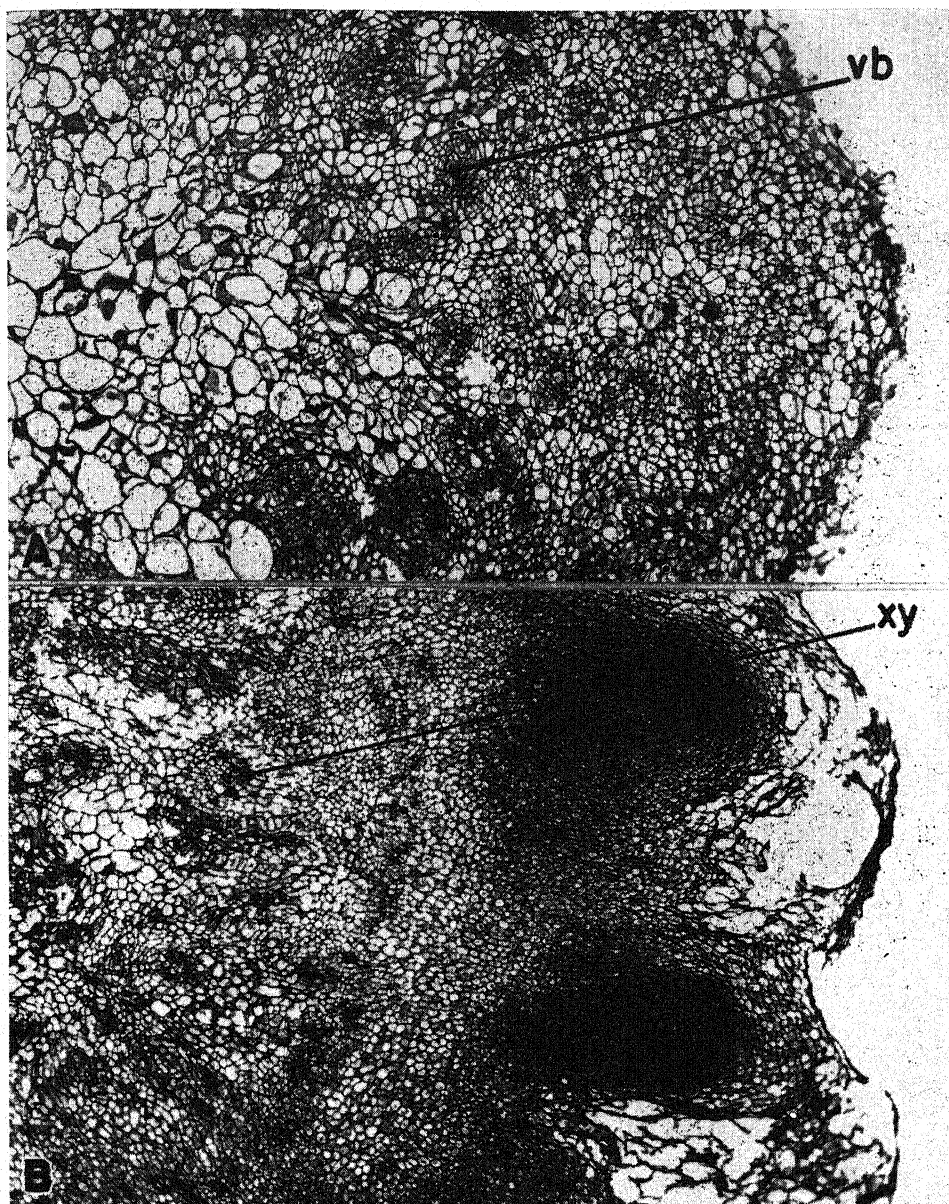


FIG. 9.—Transections of stem tip, 12 days after treatment with magnesium salt of 2,4-D. *A*, 0.5 mm. above original cut surface. Development above original cut surface characteristic of response to magnesium salt. Tumor at this level consists of parenchyma and complex system of small anastomosing vascular bundles (*vb*) and is derived mostly from proliferation of inner rays, xylem, and pith parenchyma. *B*, same stem as *A*, just below cut surface: *xy*, xylem. Marked proliferation in xylem parenchyma, inner portion of ray, and pith; derivatives maturing as parenchyma, as scattered reticulate tracheids, or as small vascular bundles. Upward proliferation of these inner tissues forms tumor above original cut surface. Differences in degree of proliferation in rays and vascular bundles more pronounced than in fig. 8*A*. Many derivatives of proliferation in vascular bundles have collapsed. Highly meristematic character of rays indicates transition to root zone. Outer tissues generally collapsed.

in the endodermis, changes in cytoplasm and nuclei of proliferated tissues preceded disorganization and collapse of the cells. This collapse progressed downward and inward into deeper layers of the tumor (figs. 2, 4C, D).

In the zone of major proliferation general maturation was longest delayed in decapitated stems treated with 2,4-D and with the ammonium, calcium, and copper salts. In these tumors the outer tissues from the epidermis to the pericycle generally collapsed to form a heavy outer layer. The wide band of proliferated tissues derived from phloem parenchyma and the outer portion of the ray continued activity as long as 2 weeks. Maturation was mainly as parenchyma. In tumors induced by the magnesium salt, maturation was established as early as 5 days. Differentiation of tracheids in proliferated endodermis and phloem parenchyma (fig. 8B) emphasized the similarity and proximity of the upper levels of these tumors to the root zone. The inner derivatives of the cambium matured as tracheids, although these were shorter and smaller than the usual xylem tracheids. An exception to generally retarded maturation was found in the proliferated parenchyma of the xylem and inner portion of the ray. Differentiation of reticulate tracheids was observed as early as 3 days in treatment with magnesium and calcium salts and 4 days with 2,4-D and the ammonium and copper salts. Other derivatives matured as parenchyma or remained meristematic and formed a complex pattern of vascular strands. Vascularization was noted with all treatments, but particularly with the copper, calcium, and magnesium salts. This type of maturation in the inner tissues extended downward through the two lower zones, gradually diminishing in extent with decreasing effectiveness of

the substance. In decapitated stems treated with the magnesium salt, and sometimes in those treated with the calcium salt, proliferation of these inner tissues at the cut surface accounted for the formation of a portion of the tumor above the original level of the cut surface. With continued development of the tumor this activity extended inward to portions of the pith (figs. 9A, B) and upward into the distinctive overgrowth at the apex of the tumor induced with the magnesium salt. In other treatments the presence of the zone of limited proliferation effectively prevented development above the cut surface.

In the root zone well-developed root tips remained meristematic until general collapse of the tips occurred. Elongation of roots was relatively slight and in no instance did the tips penetrate through the outermost tissues (fig. 11). Differentiation of the other proliferated tissues was similar to that found in the lowest zone (fig. 11A).

Relatively small areas in the zone of minor proliferation exhibited marked activity. The pattern of maturation in these areas, however, was more complex than in any other portion of the stem (fig. 15). Derivatives of endodermis and cortical parenchyma generally matured as parenchyma. Over some large bundles, particularly with the calcium and magnesium salts, reticulate tracheids, fiber-like cells, meristematic patches, and vascular bundles, as well as parenchyma, were noted (figs. 13B, 15). The reticulate tracheids in the endodermis connected through the interfascicular rays with the xylem. Maturation in the phloem resulted in large areas of reticulate tracheids intermingled with some parenchyma, scattered vascular bundles, and patches of fiber-like cells. These tracheids connected through the phloem rays with the

xylem. Frequently a band of primary phloem cells adjacent to the pericycle remained meristematic. This was particularly evident with 2,4-D and the ammonium salt but much less so with the magnesium salt (fig. 13). This complex of tissues followed no regular pattern but extended from the endodermis through the pericycle and phloem parenchyma to the cambium (fig. 15) and reached its most elaborate development at the level just below the root zone. The same pattern extended upward through the root zone to the lowest levels of the zone of major proliferation and downward with increasing simplicity to levels where apparent response ceased altogether.

Discussion

In this experiment histological responses induced in bean stems by 2,4-D and by its ammonium, copper, calcium, and magnesium salts were generally similar to those described by BEAL (1) and by SWANSON (8) for 2,4-D. SWANSON considered that each growth-regulating substance induced a pattern of reactions characteristic for that substance. He referred to this as individuality of effect. Despite certain variations in the reactions of bean plants to 2,4-D and to its salts, the responses resulting from any one treatment appeared to fall within the range of effects distinctive for 2,4-D. The differences among the tumors induced by the various substances were observed both in the histological responses and in the zonation. However, it is significant that no response induced by one substance was entirely absent in the reaction to another substance. Where differences existed there was a gradual increase or decrease in this response from one substance to the next. In general, the compounds fell into a series in the following order: at one end 2,4-D and the am-

monium salt, in an intermediate position the copper salt, and at the other end the calcium and magnesium salts.

Histological responses to the various substances differed in the following ways: (a) rate at which response was initiated and progressed to maturation; (b) degree of proliferation in various tissues and at different levels, both of which considerably influenced the shape of the tumor; and (c) the extent of maturation, determined by the number of reticulate tracheids and the amount of vascularization in proliferated tissues. Differences within the tissues may be summarized as follows:

1. Proliferation of the outer cortical parenchyma was a frequent response to the magnesium salt, although it might occur to a lesser degree over especially active bundles with any of the other substances.

2. Response in the endodermis was initiated first with the magnesium salt, next with the calcium salt, and last with 2,4-D. A similar gradation was noted in the depth of response in the endodermis (table 1). Likewise, reticulate tracheids and small vascular bundles were a common form of maturation over large bundles with the magnesium salt, although they were found to a lesser degree and more infrequently with the other substances (figs. 12, 13).

3. Divisions were observed in the inner portion of the ray and in the xylem parenchyma within 24 hours with the magnesium and calcium salts but not until 48 hours with the other substances. Maturation of derivatives as reticulate tracheids was noted on the third day with the magnesium and calcium salts but not until the fifth day with the other substances.

4. Pith parenchyma centrad to the protoxylem points was particularly active with the magnesium salt, somewhat

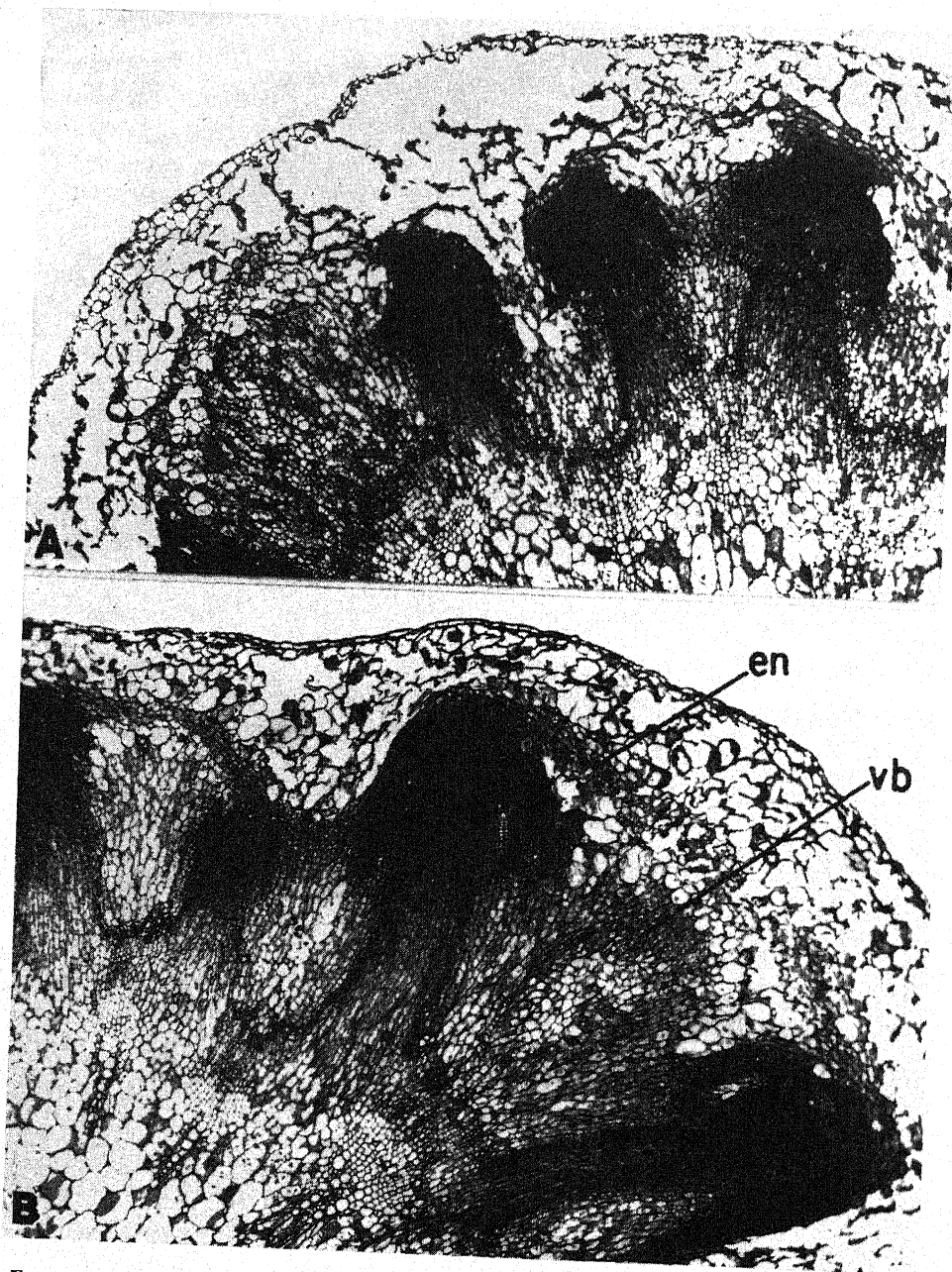


FIG. 10.—Transections of stem tips through root zone, 5 days after treatment. *A*, ammonium salt of 2,4-D, 6 mm. below cut surface. *B*, magnesium salt of 2,4-D, 3 mm. below cut surface: *en*, endodermis. In both *A* and *B* highly meristematic tissues of root primordia in rays alternating with maturing, slightly proliferated tissues of vascular bundles. Pericyclic cells enlarged over vascular bundles or maturing as fibers. Differentiation of tracheids and vascular bundles (*vb*) among derivatives of phloem parenchyma. Cells of inner portion of rays and xylem parenchyma slightly active. Except for activity in rays, degree of proliferation markedly less than in zone of major proliferation. Organization of root primordia and differentiation of proliferated cells more advanced in stem treated with magnesium salt.



FIG. 11.—Transections of stem tips through root zone, 12 days after treatment. *A*, ammonium salt of 2,4-D, 5.5. mm. below cut surface. *B*, magnesium salt of 2,4-D, 3 mm. below cut surface. More mature stages in development of root zone. Outer tissues and many areas of proliferated inner tissues collapsed.

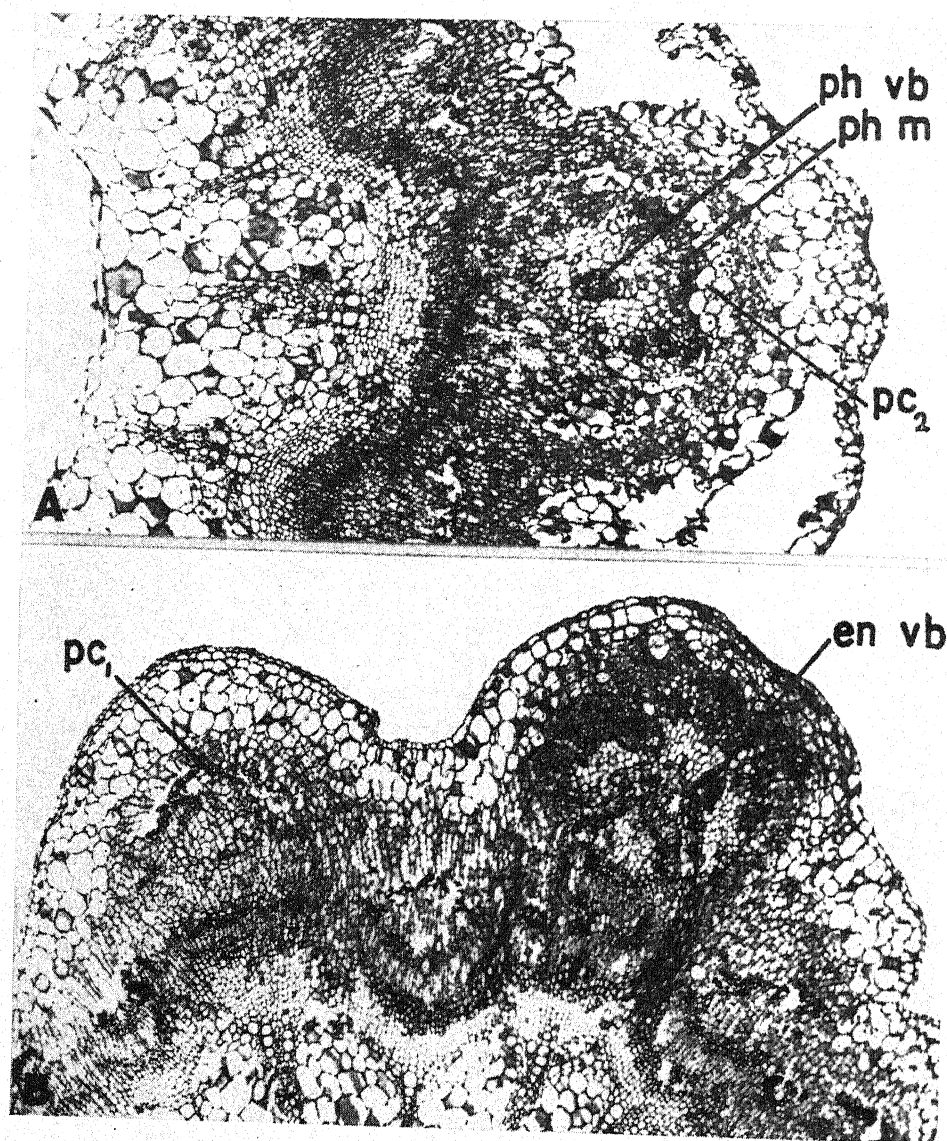


FIG. 12.—*A*, ammonium salt of 2,4-D, 7.5 mm. below cut surface; *B*, magnesium salt of 2,4-D, 5.5 mm. below cut surface. Both transections through zone of minor proliferation, 5 days after treatment. Outer cortical parenchyma generally inactive; inner cortical parenchyma and endodermis slightly proliferated and commonly maturing as parenchyma. Pericyclic cells frequently matured as fibers (*pc*₁), but some cells greatly enlarged and maturing as parenchyma (*pc*₂). Primary phloem parenchyma adjacent to pericycle remaining meristematic (*ph m*) and continuing proliferation, especially in *A*; other derivatives of proliferated phloem parenchyma maturing in complex pattern of reticulate tracheids and small vascular strands (*ph vb*). Band of cambial derivatives less wide than at upper levels, cells maturing as secondary phloem and short xylem tracheids. Slight activity in inner portion of ray, especially in *A*. Bundle to left in *B* typical of response in small bundles with both treatments. Over large bundle to right in response induced by magnesium salt outer cortical parenchyma characteristically proliferated; inner cortical parenchyma and endodermis very active with many derivatives differentiated as vascular bundles (*en vb*) or as reticulate tracheids which connect through rays to xylem.

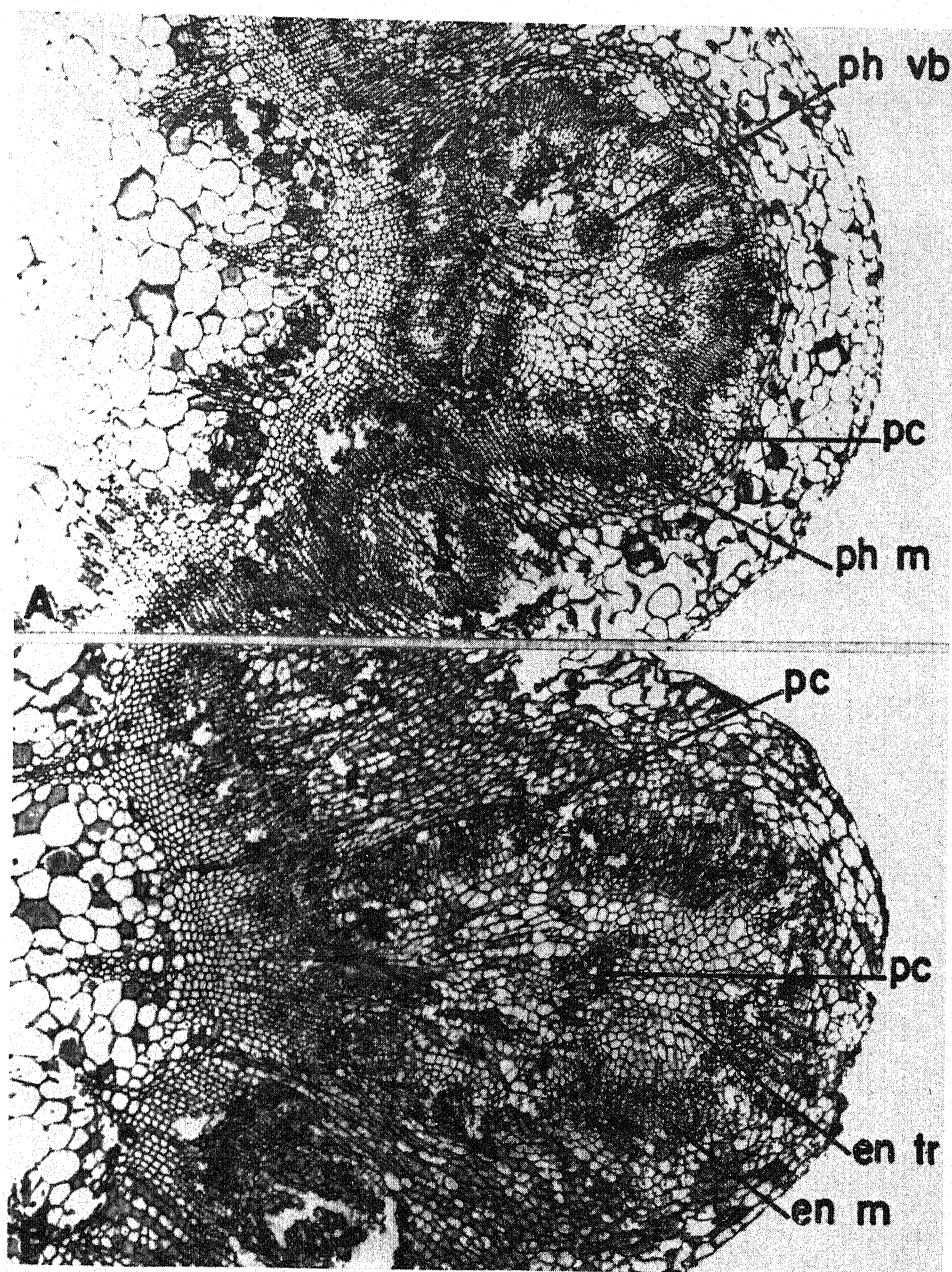


FIG. 13.—*A*, ammonium salt of 2,4-D, 8 mm. below cut surface; *B*, magnesium salt of 2,4-D, 4 mm. below cut surface. Both transections in zone of minor proliferation, 12 days after treatment: *pc*, pericycle. In general, both sections similar to those at 5 days but much more mature. In phloem and endodermis continued activity of meristematic areas (*ph m* and *en m*) and further differentiation of tracheids (*en tr*) and vascular bundles (*ph vb*) result in greater complexity of tissue patterns. Note continued proliferation of inner ray parenchyma in *A* and its absence in *B*.

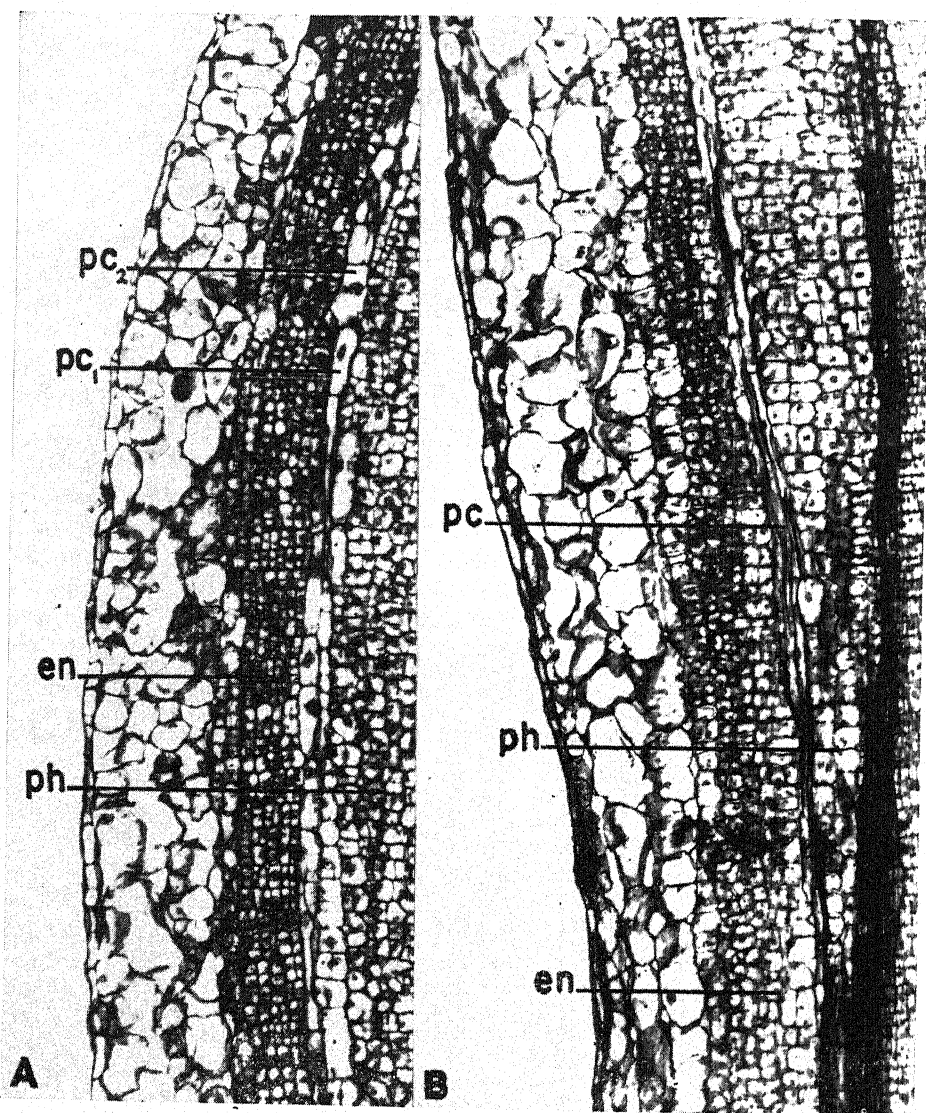


FIG. 14.—Longitudinal sections of stem tips, 3 days after treatment with copper salt of 2,4-D. *A*, detail of early proliferation at upper levels (1–3 mm.) to show response of pericyclic cells: many cells enlarged, some dividing (*pc*₁), others recently divided and multinucleolate (*pc*₂). Note character of proliferation in endodermis (*en*) and phloem parenchyma (*ph*). *B*, similar detail at lower levels (5–7 mm.). Pericyclic cells (*pc*) less active, response in phloem parenchyma (*ph*) terminating, but proliferation in endodermis (*en*) extending downward for several millimeters.

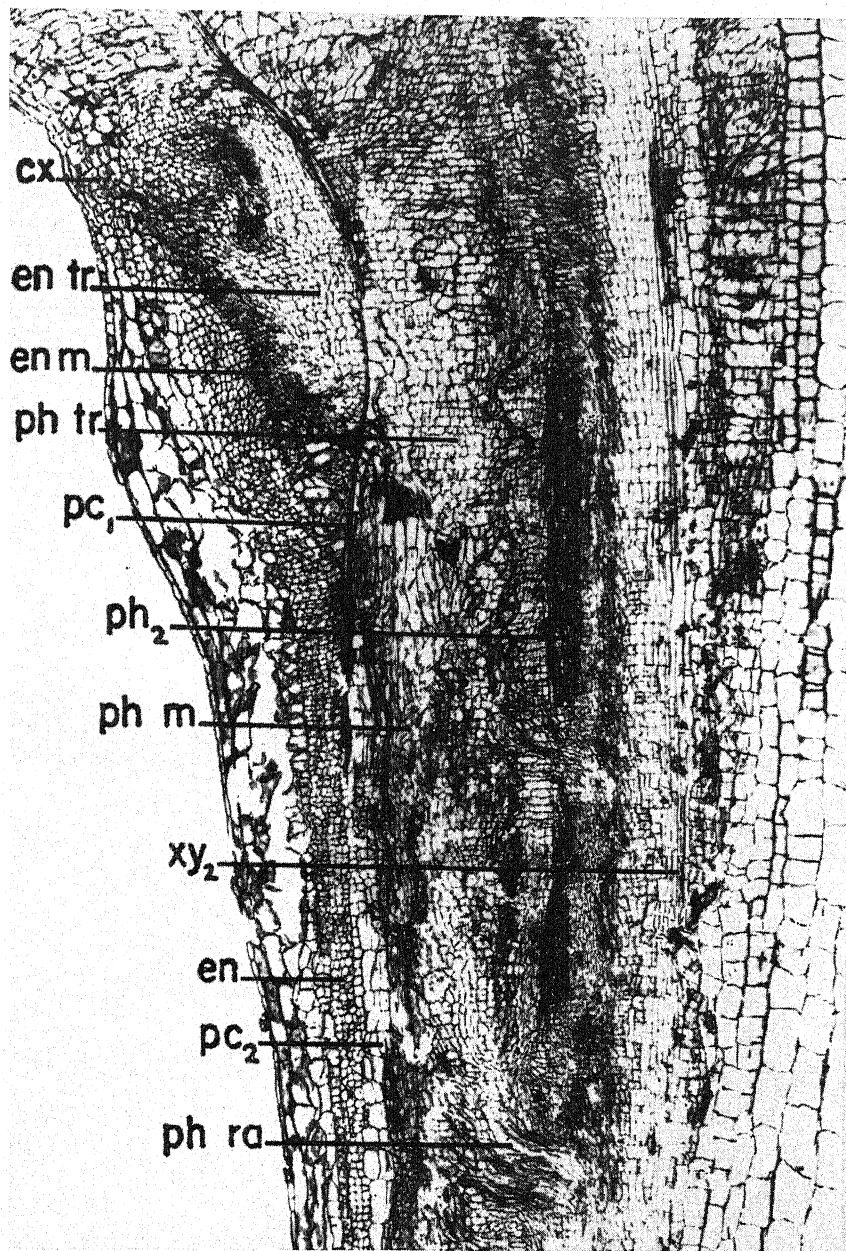


FIG. 15.—Longisection, 12 days after treatment with calcium salt of 2,4-D. Section of older stem in zone of minor proliferation (4.5–8 mm.) to illustrate in longisection complex and varying character of proliferation and maturation in large bundle. Compare with fig. 13. Cortical parenchyma (*cx*) proliferated. Endodermis strongly proliferated at upper levels and differentiating tracheids (*en tr*) or forming meristematic strands (*en m*); less active at lower levels and maturing as parenchyma (*en*). Pericyclic cells maturing as fibers (*pc₁*) or slightly proliferated and maturing as parenchyma (*pc₂*). Phloem parenchyma greatly proliferated; many derivatives maturing as tracheids (*ph tr*), strands of which may connect with xylem through phloem rays (*ph ra*); other areas parenchymatous or remaining meristematic (*ph m*). Secondary vascular tissues (*ph₂* and *xy₂*) matured.

less so with the calcium salt, and least with 2,4-D. This pith parenchyma, together with parenchymatous cells of the xylem and inner ray, proliferated above the original cut surface in tumors induced by the calcium and magnesium salts.

In the evaluation of responses induced by the various treatments zonation is an important characteristic of the tumor. A study of the tumors on the basis of zonation disclosed well-defined differences but at the same time established a consistency of response throughout all the treatments. A shallow, flared tumor with major activity just below the cut surface and some proliferation above (magnesium salt) was readily distinguished from a deep tumor with the greatest enlargement much below the cut surface and constriction at the apex (2,4-D). Although these represented extremes of response in this experiment, comparison of equivalent zones and stages of development showed close relationship between the two types. Moreover, a progressive series between these two was formed with the other salts of 2,4-D. It is significant that comparisons of the different tumors resulted in each instance in the same series of relationships among the substances, whether the differences were expressed in histological details or in the larger pattern of zonation. This is illustrated in figures 1-5 and table 2.

The series of responses strongly suggests variation in the effective concentration of the growth-regulating substances. In decapitated bean plants with a known gradation in the concentration of indoleacetic acid (7), similar changes in the character and levels of response were observed. It was shown that responses induced at successively lower levels in a stem tip treated with a high concentration of indoleacetic acid are similar to responses at higher levels induced by

treatment with lower concentrations. In the present experiment, in the stem tips treated with the magnesium salt, the responses at upper levels (0-1 mm.) were similar to those at intermediate levels (5-6 mm.) in the tumor induced by the ammonium salt (figs. 4, 5). If zonation is interpreted as an expression of concentration, the five substances used in this experiment are not equally effective. The strongest stimulation of response resulted from treatments with 2,4-D and with the ammonium salt, and the least with the magnesium salt. On this basis the zone of limited proliferation is interpreted as resulting from concentrations high enough to approach toxicity and to inhibit growth. The zone of major proliferation represents a concentration of greatest effectiveness in stimulating and prolonging growth. Roots appear to be induced at lower concentrations. Minor proliferation occurred at still lower levels and concentrations. A somewhat similar series of effects from stimulation to inhibition of growth correlated with low and high concentrations of indoleacetic acid is reported by HSUEH and LOU (4).

Explanation of the apparent concentration gradient with the different salts of 2,4-D is difficult. All substances were applied in 0.5% concentration by weight. Differences in the molecular weights of the compounds result in only slight variation in the amount of 2,4-D present and do not appear to offer an explanation, particularly as the sequence in the molecular weights does not correlate with the sequence established for effectiveness of response. Solubility was also taken into consideration but did not seem to be a direct factor. The method by which the cation changed the effective concentration and thus modified the activity of the growth-regulating substance is difficult

to analyze. Reduction in the effectiveness of the various substances may take place in numerous ways, such as the initial entrance of the substance into the plant tissues, translocation, changes in the permeability of the membranes, and the reactions within the cells.

The role of the cation in directly influencing the character of histological response is also difficult to determine. Certain histological differences may be attributable to independent action of the cation or these differences may be correlated with zonation and concentration and thus indirectly with the cation. How-

ever, much of the response appears to be a 2,4-D effect. The result of greatest significance in the present experiment is the considerable reduction in the effective concentration of the growth-regulating substance according to the kind of salt of 2,4-D in which it is applied.

Summary

1. Young kidney-bean plants were decapitated in the second internode. 2,4-Dichlorophenoxyacetic acid or one of four salts—ammonium, copper, calcium, or magnesium 2,4-dichlorophenoxyacetate—was applied to the cut surface in

TABLE 2

ZONATION IN TUMORS INDUCED BY 2,4-DICHLOROPHENOXYACETIC ACID
AND FOUR OF ITS SALTS, 12 DAYS AFTER TREATMENT

Depth in stem (mm.)	2,4-D	Ammonium salt	Copper salt	Calcium salt	Magnesium salt
Cut surface					
0	Limited proliferation	Limited proliferation	Limited proliferation	Major proliferation	Major proliferation
1					
2			Major proliferation		Root formation
3		Major proliferation		Root formation	
4	Major proliferation		Root formation		Minor proliferation
5		Root formation	Root formation	Minor proliferation	
6	Root formation		Minor proliferation		
7		Minor proliferation			
8	Minor proliferation				
9					
10					
11					
12					
13					
14					
15					

0.5% concentration by weight in lanolin paste. Observations were made of material grown for a period of 30 days after treatment.

2. Gross response showed characteristic differences among the tumors. Treatments with 2,4-D and with the ammonium salt resulted in relatively deep, club-shaped tumors. Proliferation was entirely below the cut surface. The tumor induced by the copper salt was likewise formed below the cut surface but was somewhat shallower. Those induced by calcium and magnesium salts were broadly flared at the cut surface and gradually tapered below. Growth above the cut surface occurred with both of the latter substances but to a more marked degree with the magnesium salt.

3. Histological responses in the early stages showed considerable similarity. In parenchymatous tissues of the inner cortex including the endodermis, of the pericycle, phloem, cambial zone, xylem, and rays, proliferation was initiated soon after treatment. Response induced by the magnesium salt was earlier and more marked than in other treatments, less so with the calcium salt, and least with the ammonium salt and with 2,4-D.

4. Study of the tumor as a whole disclosed a pattern of zonation for all treatments. At upper levels a zone of limited proliferation was found in the responses to 2,4-D and to the ammonium and copper salts. This zone was lacking in the other responses. A zone of major proliferation occupied the intermediate levels below the zone of limited proliferation in treatments with 2,4-D and with the ammonium and copper salts but was found at upper levels in the tumors induced by the calcium and magnesium salts. In response to the magnesium salt this zone was only poorly represented. With all

substances a root zone was formed at next lower levels, and below the roots a zone of minor proliferation terminated the tumor. Comparison on the basis of zonation disclosed a consistent sequence among the substances according to the characteristics of the responses induced: 2,4-D and the ammonium, copper, calcium, and magnesium salts. This same series was duplicated in gross responses and in details of histological responses.

5. Maturation was earliest and most marked in the treatment with the magnesium salt and least with the ammonium salt and with 2,4-D. In the zone of limited proliferation maturation occurred early, was mainly as parenchyma, and was soon followed by collapse. Maturation in the zone of major proliferation was longest delayed. Many of the derivatives differentiated as parenchyma except at levels of transition to lower zones. In the root zone the rays organized as root primordia remained active until the death of the tumor 3 weeks or more after treatment. Proliferated tissues of vascular bundles and associated endodermis and cortical parenchyma both in the root zone and in the zone of minor proliferation exhibited complicated patterns of differentiation into tracheids, parenchyma, vascular bundles, and meristematic areas.

6. Characteristic differences in histological responses to the different substances are discussed. The most pronounced differences were found in decapitated stems treated with the magnesium salt. However, no response induced by one substance was entirely absent in the reaction to another substance. All responses appeared to fall within the range of effects distinctive for 2,4-D. Independent effects of the different cations on stem responses were also considered.

7. Zonation is interpreted as an expression of a concentration gradient. On the basis of this interpretation the effectiveness of the growth-regulating substance

was greatly modified by the form of the salt which was applied.

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A RAPID SENSITIVE METHOD FOR DETERMINATION OF LOW CONCENTRATIONS OF 2,4-DICHLOROPHENOXY-ACETIC ACID IN AQUEOUS SOLUTION¹

DANIEL READY² AND VIRGINIA Q. GRANT³

Introduction

In physiological and herbicidal studies on 2,4-dichlorophenoxyacetic acid (2,4-D) there is frequently a need for determining the biological activity of small amounts of this compound in aqueous solution. No satisfactory chemical method has yet been devised, primarily because this compound is relatively inert chemically.

¹ Studies conducted at Camp Detrick, Frederick, Maryland, from April to June, 1946.

² Biochemist; ³ botanist.

Recently BANDURSKI (1) described a spectrophotometric method for determining 2,4-D in aqueous and ether solutions in which Beer's law was found to hold within 5% limits over a concentration range of 6-250 γ /ml. This method is based on the absorption of light in the ultraviolet region. Earlier SWANSON (3) devised a bio-assay method based on the inhibition of further growth of the primary root of surface-sterilized and pre-germinated corn seeds. Within the limits of 0.1-2.0 p.p.m., reproducible and reasonably accurate results were obtained.

The sensitivity of other germinating seeds to 2,4-D has been examined in an attempt to extend the range of application of the bio-assay procedure based on this principle. Representative species of the families Leguminosae, Compositae, Gramineae, Cucurbitaceae, Liliaceae, Malvaceae, and Polygonaceae were tested, and it was found that a member of the Cucurbitaceae, the common cucumber (*Cucumis sativus*), is very sensitive to 2,4-D. A detailed study was therefore made of the relationship between the degree of primary root and shoot inhibition of germinating cucumber seed and small concentrations of 2,4-D; the results form the basis of this paper.

Material and methods

The cucumber seed used was a commercially available variety, Early Fortune. Six-inch Petri dishes were fitted with circles of filter paper and to each were added 15 ml. of the solution of 2,4-D under test. Twenty-five cucumber seeds were placed in each dish, and five replicate dishes were employed for each of fifteen different concentrations. Untreated controls were set up using 15 ml. of distilled water. The dishes were then covered and placed in a dark constant-temperature room at 28° C. At the end of 96 hours they were removed, and lengths of the primary root and shoot were measured.

Results

The concentrations employed ranged from 0.001 to 10.0 p.p.m. Examination of the data (tables 1, 2, figs. 1-3) shows that there was a simple quantitative relationship between the concentrations of 2,4-D used and the degree of inhibition of growth of primary roots and shoots of the germinating cucumber seed. Calculation

of the F values for these data showed high statistical significance for treatment and no significant difference for replication.

The roots were extremely sensitive to minute amounts of this acid, concentrations as low as 0.005 p.p.m. having produced inhibition to a degree which was statistically significant when compared with the control (table 1). All concentrations above this point to the maximum of 10 p.p.m. produced growth inhibition highly significant at the 1% level when compared with the control. Differences between treatments were also highly significant at concentrations from 0.01 to 0.10 p.p.m. At a concentration of 1.0 p.p.m. inhibition of growth had essentially reached its maximum, and the curve leveled off. Consequently, inhibition of growth of the primary root offers an excellent means of determining 2,4-D in concentrations of 0.005-1.0 p.p.m.

The shoots treated with 0.01 p.p.m. (table 2) showed appreciable growth inhibition when compared with the control, but the differences between treatments were not statistically significant until a concentration of 0.50 p.p.m. was reached. Differences between treatments were highly significant between 0.5 and 5.0 p.p.m. with the exception of the 1.0 p.p.m. concentration. Above 5.0 p.p.m. maximum inhibition of shoot development had been practically attained, and the curve leveled off. Consequently, measurement of the shoot offers an additional means for determining concentrations of 2,4-D from 0.5 to 5.0 p.p.m.

When the values for root and shoot inhibition were plotted against log concentrations of 2,4-D, straight lines were obtained for which equations fitting closely were derived (fig. 3). The slopes of the lines are not quite identical.

TABLE 1
INHIBITION OF PRIMARY ROOTS OF GERMINATING CUCUMBER SEEDS EXPOSED FOR
96 HOURS TO DIFFERENT CONCENTRATIONS OF 2,4-D

CONCENTRATION OF ACID (P.P.M.)	MEAN LENGTHS IN REPLICATE (MM.)					MEAN*	% OF CONTROL
	1	2	3	4	5		
0 (control).....	70	62	58	67	57	62.8
0.001.....	60	66	60	55	59	60.0	95.5
0.005.....	57	61	56	62	55	58.2	92.6
0.010.....	44	37	49	45	42	43.4	69.1
0.025.....	38	35	36	36	42	37.4	59.5
0.050.....	29	33	27	28	34	30.2	48.0
0.075.....	23	26	26	27	24	25.2	40.1
0.10.....	14	18	20	18	29	19.8	31.5
0.25.....	18	15	17	18	16	16.8	26.7
0.50.....	12	13	12	13	14	12.8	20.3
0.75.....	9	10	12	11	10	10.4	16.5
1.0.....	8	8	13	9	10	9.6	15.2
2.5.....	7	8	6	7	9	7.4	11.7
5.0.....	6	7	6	7	7	6.6	10.5
7.5.....	6	7	6	7	6	6.4	10.1
10.0.....	6	6	6	6	6	6.0	9.5

* Minimum significant difference between means of concentrations at 5% level of probability is 3.82 mm.; at 1% level, 5.08 mm.

TABLE 2
INHIBITION OF SHOOTS OF GERMINATING CUCUMBER SEEDS EXPOSED FOR 96 HOURS
TO DIFFERENT CONCENTRATIONS OF 2,4-D

CONCENTRATION OF ACID (P.P.M.)	MEAN LENGTHS IN REPLICATE (MM.)					MEAN*	% OF CONTROL
	1	2	3	4	5		
0 (control).....	60	56	53	60	59	57.6
0.001.....	53	53	56	55	57	54.8	95.1
0.005.....	52	54	50	56	54	53.2	92.3
0.010.....	50	52	49	50	47	49.6	86.1
0.025.....	49	51	46	53	55	50.8	88.1
0.050.....	45	52	45	50	54	49.2	85.4
0.075.....	52	48	47	48	55	50.0	86.8
0.10.....	53	47	56	51	48	51.0	88.5
0.25.....	50	45	48	41	50	46.8	81.2
0.50.....	44	43	39	47	44	43.4	75.3
0.75.....	38	37	37	38	38	37.6	65.2
1.0.....	37	33	37	40	37	36.8	63.8
2.5.....	31	28	27	26	28	28.0	48.6
5.0.....	18	23	20	22	19	20.4	35.4
7.5.....	14	15	15	18	17	15.8	27.4
10.0.....	13	14	16	14	13	14.0	24.3

* Minimum significant difference between the means of concentration at 5% level of probability is 3.34 mm.; at 1% level, 4.44 mm.

Discussion

The need for a sensitive and accurate method for the determination of small amounts of 2,4-D in aqueous solution has been adequately met through the activity of this acid in inhibiting the primary root and shoot of germinating cucumber

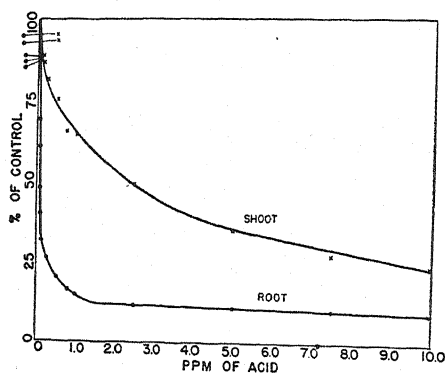


FIG. 1.—Inhibition of growth of primary roots and shoots of germinating cucumber seed exposed for 96 hours to different concentrations of 2,4-D.

seed. Such activity is so quantitatively related to the concentrations of acid employed that this fact serves as a means of determining the amounts of acid present. Differing sensitivities of the root and shoot to the same concentration of the acid present a desirable condition whereby, in the case of an unknown solution, either the root or the shoot, or both in the case of the 0.50–1.0 p.p.m. range, may be used to determine the acid concentration.

Furthermore, this method is simple and rapid. No surface sterilization of the seed was found to be required, since in this study and in hundreds of other determinations fungal contamination was very infrequent, even though untreated seed only was used. In addition, no initial germination and subsequent selection of the seed are necessary before treatment as in the method described by SWANSON

(3). It can be performed with a minimum of equipment and could undoubtedly be employed in the field, provided a reasonably constant-temperature storage chamber is available near by. By measuring the activity on germinating cucumber seeds, concentrations from 0.005 to 5.0 p.p.m. can be determined accurately by this method, and dilution to this range will permit determination of more concentrated solutions. Solutions of 2,4-D of unknown concentration, after dilution, may be subjected to the procedure herein described, and growth inhibition may be expressed in percentage of control. This may then be compared with the standard curve, and the presumptive concentration of 2,4-D may be thereby ascertained.

The linear character of the relationship between inhibition of both roots and shoots of the cucumber seedling and concentrations of 2,4-D considered on a

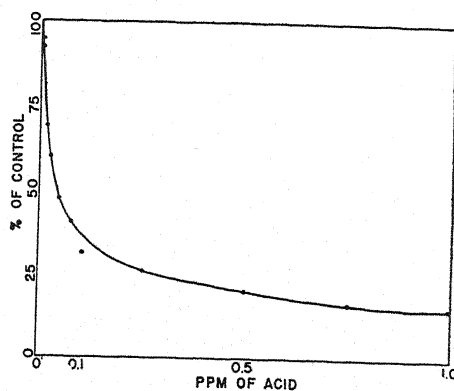


FIG. 2.—Inhibition of growth of primary roots of germinating cucumber seed exposed for 96 hours to different concentrations of 2,4-D.

logarithmic basis may throw some light on the mechanism of action of a growth-regulating substance of this type. It is not known whether the over-all inhibition produced is the result of a simple effect on some cellular mechanism, or

whether it is a summation of effects on various growth mechanisms. The latter is the more probable, since the magnitudes of the responses produced in root and shoot are not identical. However, as far as each organ is concerned, the effect is proportional to concentration, and the relationship can apparently be treated as a single reaction.

The procedure thus developed is predicated entirely upon the activity of 2,4-D on germinating cucumber seeds in aqueous media. Recently LUCAS and HAMNER (2), using onion extract, found that activation of 2,4-D was obtained through a synergistic effect; they also found that dilution of the extract increased this synergistic effect. Some lesser synergistic effect was evidenced in the case of garlic extract, whereas none was found with tomato extract. In work under progress at this installation, inhibition of germinating cucumber seed by some factor present in corn-plant extract has been noted. On the other hand, soil leachates have been examined for 2,4-D content by this method, and, after suitable standard curves had been established by adding known amounts of 2,4-D to the leachate from untreated soil, good agreement was obtained between the theoretical amounts of 2,4-D added to the soils and recovery. Also, determinations on artesian waters to which known amounts of 2,4-D had been added were in good agreement with the standard curve. From the foregoing it is obvious that in certain types of biological materials the terms "activity" (or inhibition) and "concentration" may not be used synonymously, and, consequently, application of the procedure described in this paper would be relative rather than absolute. The basic purpose of this paper, however, is to describe a quantitative method for the determina-

tion of 2,4-D in aqueous solutions which can then be modified as required for the determination of this acid in other media, such as by constructing standard curves using such media as a base, or by the inclusion of appropriate blanks.

Further investigations are under way to determine the suitability of this method for the quantitative estimation of ac-

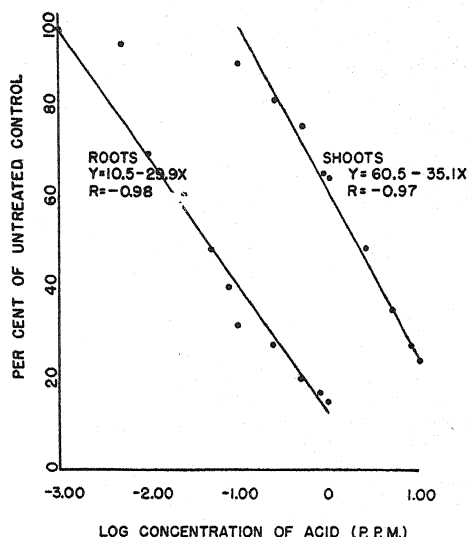


FIG. 3.—Regression equations and coefficients of correlation of roots and shoot lengths of germinating cucumber seed exposed to 2,4-D.

tivity of small amounts of other members of the phenoxyacetic acid series (4). Included in this group are 4-chlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid. Preliminary results appear encouraging and publication of results of detailed tests on these compounds will be made shortly.

Summary

1. A simple and rapid quantitative method for the determination of small amounts of 2,4-dichlorophenoxyacetic acid (2,4-D) in aqueous solution is de-

scribed. This method is based on the activity of the acid in inhibiting the growth of the primary root and shoot of germinating cucumber seed. It is sensitive to 0.005 p.p.m. and is applicable from

this concentration to 5.0 p.p.m. Initial surface sterilization of the seed, selection of pregerminated seed of certain root lengths, and special equipment are not necessary.

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VEGETATIVE RESPONSES OF *BROMUS INERMIS* TO CERTAIN VARIATIONS IN ENVIRONMENT¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 587

JOHN C. DIBBERN

Introduction

Smooth brome grass (*Bromus inermis* Leyss.) has long been cultivated in Hungary and Russia. The first recorded importation of seed into the United States was in 1880, and many importations from different sources have been made since. From these importations our present strains have been developed.

The species is now found as far south as Tennessee, Kansas, and California and extends northward through Canada into Alaska. On mountain ranges in the semiarid regions of the West and Northwest it is frequently found at all elevations up to 9,000 feet, and in central Utah up to 10,500 feet, though it does not reseed at this elevation (3). It is important as a hay and pasture grass, especially from Minnesota and Kansas to eastern Oregon and Washington, occasionally

eastward to Michigan and Ohio; and in the artificial reseeding of denuded range land in regions of light rainfall and moderate summer temperatures.

Smooth brome is a perennial and spreads vegetatively by means of rhizomes. It grows rapidly in the spring and, though remaining green throughout the warm summer months, then makes very little additional growth. Inflorescences develop in the spring, and anthesis occurs in early summer. Through environmental selection northern and southern strains have evolved, these strains maturing late and early, respectively, when grown in the latitude of Lincoln, Nebraska (8). Within these strains the individual plants show a great diversity of growth habit, ranging from bunch types to semicreepers.

A study has been made of some of the vegetative responses of diverse plants of smooth brome grass to variations in clipping frequency, soil temperature,

¹ This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

shading, and container size in an attempt to shed some light on (a) the ability of the species to withstand grazing or mowing; (b) its limitation in distribution to only the higher latitudes; (c) its shade tolerance; and (d) the type of container that will allow maximum growth in the greenhouse. The experiments were conducted in the greenhouses and garden of the University of Chicago from the spring of 1946 through early summer, 1947.

Material and methods

Because of the diversity of the species, it seemed desirable to work with a wide variety of plants. To this end the clonal and seed materials used represent plants originating from Saskatchewan to Washington, Kansas.

In greenhouse experiments the soil used consisted of three parts loam soil of prairie origin mixed with one part sand. A supplementary nitrate solution was occasionally applied and consisted of 3.5 gm. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ per liter of water.

In experiments where both root and top weights are reported, the separation of tops and roots was made on a morphological basis rather than at the ground level. Underground stem parts were thus considered part of the tops. Where root weights were not taken, tops were clipped at a height of 2 inches above the ground level except where specifically stated otherwise.

Stem counts represent only those stems that had emerged from the soil, including both elongated and unelongated tillers, but do not include dead stems.

Experimentation

CLIPPING

GREENHOUSE SERIES.—On April 27 and 28, 1946, six divisions of each of twenty-three clones which had been

growing outdoors either at Chicago, Illinois, or at Madison, Wisconsin, were planted separately in 8-inch unglazed clay pots on well-illuminated benches in the greenhouse. Each division consisted of approximately six stems and accompanying roots. The leaves of plants that showed severe wilting after planting were clipped to decrease water loss, but in no instance was the clipping severe enough to remove entire leaf blades or growing points. Nitrate solution (192 ml.) was supplied to each plant on May 4, 1946, September 5, 1946, and April 3, 1947, and they were watered when necessary.

On June 30, 1946, the three most uniform divisions of each clone were selected for experimentation, but only the seventeen clones in which all three divisions survived until the termination of the experiment are reported upon. Series C was an unclipped control, series 1 was clipped on June 30, 1946, and series 2 on June 30 and September 27.

Because of general lack of vigor, a final harvest was considered inadvisable in the fall, and the effects of clipping for the 1946 season are therefore shown only by stem counts (table 1).

The clipping on June 30 caused a reduction in the number of stems in series 1 to 76% as measured on July 13, but series 2 was reduced to only 86%. The stem counts and top weights on June 30 were larger in series 2 than in series 1, and it would thus appear that the larger plants were less affected by clipping than were the smaller ones.

On September 27 the stem count of series 1 was 2% below the count on June 30, and that of series 2 was 23% above. The yield of tops in series 2, however, was less than half that of June 30. The lower yield between June 30 and September 27 cannot be considered entirely an effect of the first clipping. Growing con-

ditions in the greenhouse during this period were quite adverse. Daytime soil temperatures averaged 23° C., and maximum air temperatures 33° C. The plants also became infested with mealy bugs, aphids, and red spiders, and, in addition to the injuries so caused, there was some injury from control spraying.

On November 24, stem counts showed the control to have increased 97% over the value on June 30, series 1 had in-

size, as suggested by the results with different-sized containers (see soil-volume experiment). On April 25 the three series were clipped back to 2 inches. The relative yields produced between April 25 and June 2, and the relative numbers of stems on June 2, 1947, when the experiment was terminated, are taken as a measure of the aftereffects of no clipping, one clipping, and two clippings the previous year. If the top growth produced

TABLE 1
STEM COUNTS AND DRY WEIGHTS OF TOPS IN GREENHOUSE CLIPPING EXPERIMENT

SERIES	1946				1947			1946		1947	
	6/30	7/13	9/27	11/24	1/18	3/31	6/2	6/30	9/27	4/25	6/2
	No. of stems*							Dry wt. of tops (gm.)*			
C.....	292	378	530	575	559	540	527	46.19	35.20
1.....	246	186	241	276	270	316	290	59.14	30.59	22.87
2.....	305	263	375	280	269	278	282	74.46	35.28	19.94	20.71
	No. of stems in % of count on 6/30							Dry wt. per stem (gm.)			
C.....	100	129	182	197	192	185	180	0.086	0.067
1.....	100	76	98	112	110	128	118	0.240097	.079
2.....	100	86	123	92	88	91	92	0.244	0.094	0.072	0.073

* Sums of values for seventeen clones.

creased 12%, and series 2 had decreased 8%. During the period from November 24, 1946, to January 18, 1947, the light intensity was quite low in the greenhouse, and all three series showed a decrease in the number of live stems.

By March 31, 1947, the plants had resumed active growth, and both clipped series showed an increase in stem numbers over the January count. However, the stem count of series C showed a decrease from the January count. This decrease might indicate that the volume of soil in the 8-inch clay pots had become a limiting factor to further increase in

between April 25 and June 2 is converted to dry-weight yield per stem based on the counts of June 30, 1946, the value for series 1 is 23% less, and that of series 2 is 43% less, than the control. The stem counts on June 2, 1947, show that series C had 80% more and series 2 had 8% less than on June 30, 1946.

HARRISON and CRAWFORD (5) found in a field experiment with smooth brome that applications of ammonium sulfate had more effect upon stem size than upon total number of stems in determining yield of forage. The total dry-weight yields in this clipping experiment, how-

ever, showed much closer correlation with stem counts than with average weight per stem (table 1).

GARDEN SERIES.—On April 25, 1946, six divisions of each of twenty-two clones were planted in six plots laid out side by side in an east-west direction in the garden. Each division consisted of approximately six stems and their roots and was taken from plants growing outdoors either at Chicago, Illinois, or at Madison, Wisconsin. The leaves of plants that showed severe wilting after planting were clipped to decrease water loss, but in no instance was this clipping severe enough to remove entire leaf blades or growing-points. The divisions were spaced 18 inches apart in the east-west direction and 24 inches apart in the north-south. The plants were watered sufficiently to prevent deficiency at any time. On June 24, 1946, the three most uniform divisions of each clone were selected for the clipping experiment.

Series C was an unclipped control, series 1 was clipped on June 24, and series 2 on June 24 and August 23. On November 15 all three series were clipped back to 2 inches and the total oven-dry yield of tops was determined. On June 7, 1947, the three series were clipped again to 2 inches, and the relative dry-weight yields were used as a measure of the aftereffects of clipping treatments in the preceding year.

The first stem count was made on June 24, 1946, at the time series 1 and series 2 were clipped the first time. The larger dry weight of tops which had been produced by series 2 was accounted for partially by the greater number of stems and partially by the greater weight per stem (table 2).

On August 23, when series 2 was clipped a second time, the numbers of stems in series 1 and series 2 were sub-

stantially greater than the numbers on June 24, though less than in the control. In contrast to the low yield from the second clipping of plants in the greenhouse, the second clipping in the garden yielded several times as much dry matter as the first, and the weight per stem was over 50% greater (table 2). This contrast is quite sharp and, even though there were five additional clones included in the garden experiment, emphasizes the differences between growing conditions outdoors and in the greenhouse.

On November 15 a stem count was made, and all three series were clipped to determine the total yields in 1946. The relative stem count was 27% less in series 1 and 55% less in series 2 than the control. The weight per harvested stem was 5% less in series 1 and 43% less in series 2 than the control. The total top weight per original stem (based on count of June 24) was 24% less in series 1 and 30% less in series 2 than the control.

From the clipping of June 7, 1947, the dry-weight yield per original stem (count of June 24, 1946) was 21% less in series 1 and 56% less in series 2 than the control.

Although these plants which grew outdoors made a much more vigorous growth than those in the greenhouse (tables 1 and 2), as measured by stem counts, top yields, and individual stem weights, the aftereffects of clipping the preceding season are very similar in greenhouse and garden as measured by the percentage differences between the 1947 yields per stem (count of June 24 and June 30, 1946) in series C, series 1, and series 2.

SEEDLINGS.—On April 30, 1947, five 8-inch glazed pots were planted with caryopses of a southern strain (no. 25); five with a strain of intermediate latitude (no. 27); and five with a northern strain (no. 26). The caryopses were covered

with $\frac{1}{4}$ inch of sifted soil, and the pots were placed on a well-illuminated bench in the greenhouse. The seedlings were watered whenever necessary to prevent water deficit and were thinned to ten per pot on May 27. On June 2, one pot (no. 1) of each strain was harvested, and oven-dry top and root weights were determined. On the same day the plants in pots 2, 3, and 4 of each strain were

This point is emphasized, since it is pertinent to the effects of clipping upon top and root development.

Pot no. 2 of each strain, harvested 6 days following clipping on June 2, showed a higher T/R ratio in the southern and northern strains, but a lower T/R ratio in the central strain, than pot no. 1, which was harvested without being clipped (table 3).

TABLE 2
STEM COUNTS AND DRY WEIGHTS OF TOPS IN GARDEN CLIPPING EXPERIMENT

SERIES	1946				1947	1946				1947
	6/24	7/13	8/23	11/15	5/12	6/24	8/23	11/15	Total	6/7
	No. of stems*					Dry wt. of tops (gm.)*				
C.....	357	665	1896	8401	8127	1768.66	1768.66	3607.5
1.....	302	344	1169	5213	5252	72.43	1062.03	1134.46	2412.5
2.....	434	505	1769	4675	4784	129.17	828.79	550.36	1508.32	1965.0
	No. of stems in % of count on 6/24					Dry wt. per stem (gm.)				
C.....	100	186	531	2375	2276	0.21
1.....	100	114	386	1726	1739	0.2420
2.....	100	116	408	1077	1102	0.30	0.47	0.12

* Sums of values for twenty-two clones.

clipped back to 4 inches high. On June 8, pot no. 2 was harvested and pot no. 3 was again clipped back to 4 inches. On June 14 the remaining pots (nos. 3, 4, and 5) were harvested, and oven-dry top and root weights were obtained (table 3).

In all three strains the top-weight/root-weight ratio (T/R) of unclipped seedlings harvested on June 2 was greater than for unclipped seedlings harvested 12 days later (pots 1 and 5). The increase in root weight in proportion to top weight as the seedling matures results from the increasing synthesis of carbohydrate by the photosynthetic tissue, which appears to have first priority on the seed reserves.

Plants clipped twice (pot 3) and once (pot 4) before harvesting produced less total top weight than the control (pot 5). The effect of clipping the tops in limiting the growth of roots was even greater than it was upon the tops and is reflected in the T/R ratios. In strains 25 and 27 the root weight in pot 3 harvested on June 14 is approximately the same as in pot 1 harvested on June 2—no net increase in root weight in 12 days when the tops were clipped twice.

DISCUSSION.—The results of both the greenhouse and the garden clipping experiments show conclusively that both the top yield per original stem and the num-

ber of stems were inversely proportional to the frequency of clipping and that these effects were carried over into the following growing season. With *Bromus inermis* HARRISON and HODGSON (6) also found that frequent and close clipping reduced both the amount of root growth and the total yield of tops and that, when the plants were completely defoli-

carbohydrate reserves was influenced less by the frequency than by time and degree of clipping. The period of reproduction from flower-stalk elongation to formation of ripe seed is critical, and clipping at this time seems to limit subsequent storage.

The direct cause of the limitation of top growth resulting from clipping is re

TABLE 3
DRY WEIGHTS OF TOPS AND ROOTS AND T/R RATIOS FOR SEEDLING
CLIPPING EXPERIMENT*

STRAIN	POT NO.	TOP WT. (GM.)				ROOT WT. (GM.)	T/R
		6/2	6/8	6/14	Total	6/14	
25.....	1	0.55	0.55†	0.12†	4.6†
	2	0.35‡	0.47	0.82§	0.14§	5.9§
	3	0.30‡	0.13‡	0.37	0.80	0.12	6.7
	4	0.30‡	0.83	1.13	0.22	5.1
	5	1.89	1.89	0.61	3.1
27.....	1	0.64	0.64†	0.12†	5.3†
	2	0.36‡	0.53	0.89§	0.18§	4.9§
	3	0.35‡	0.18‡	0.41	0.94	0.13	7.2
	4	0.38‡	0.84	1.22	0.26	4.7
	5	2.17	2.17	0.62	3.5
26.....	1	0.30	0.30†	0.07†	4.3†
	2	0.22‡	0.48	0.70§	0.13§	5.4§
	3	0.20‡	0.16‡	0.49	0.85	0.20	4.2
	4	0.21‡	0.67	0.88	0.22	4.0
	5	1.46	1.46	0.52	2.8

* Values are sums of ten plants in each pot.

† On June 2.

‡ Clipped to 4 inches.

§ On June 8.

ated, new top growth was developed in a large measure at the expense of previously deposited root reserves. McCARTY and PRICE (7) found that the quantity of reserve carbohydrate stored in roots and stem bases of mountain brome and slender wheatgrass growing on the Wasatch Plateau of Utah was related to the amount of foliage present during the normal storage period of August and September and was less when the interval between earlier clipping and the normal storage period was shortened. Their results also indicated that the amount of

lated primarily to the reduction in photosynthetic tissue and to the use of reserves in the growth of new tops but is probably also markedly influenced by the concomitant limitation on root growth. The seedling experiment in the greenhouse showed that root growth was even more severely affected by clipping than was top growth. ROBERTSON (9) also found with *B. inermis* that, as a result of repeated clipping of tops, the rate of root growth diminished gradually until it ceased entirely, followed by a dying back from the root tips.

The findings of numerous workers (1, 2, 12) indicate that the greater yield of unclipped grass over periodically clipped grass is largely of fiber and other nitrogen-free substances. However this may affect the feeding value of the forage, one must not lose sight of the fact that the effects of clipping upon root growth and upon storage of reserves do govern over a period of years a perennial plant's ability to withstand adverse environmental conditions. This is brought out in the contrast between the stem counts in the garden experiment (table 2) and the greenhouse experiment (table 1). The general lack of vigor of the plants in the greenhouse clipping experiment which were also subjected to high temperatures and low light intensity lends stress to the point made by GRABER (4) that the productive capacity of grasses depends upon adequate supplies of available nutrients and moisture combined with favorable light and temperature conditions as well as upon adequate food reserves.

SOIL TEMPERATURE AND CLIPPING

In an attempt to determine the effects of soil temperature combined with clipping upon different types of brome grass, clone 4, a creeper from Washington; clone 11, a Parkland bunch type; clone 25, a southern type, with origin at Superior, Nebraska; and clone 26, a northern type with origin at Mandan, North Dakota, were selected for experimentation. The divisions of clones 4 and 11 were made by slicing uniform-sized root segments and accompanying stems from uprooted plants that had been established in the garden; those of clones 25 and 26 by slicing into uniform segments plants that had been established in 6-inch pots in the greenhouse. The root mass of the divisions of each clone was relatively uniform, but the accompany-

ing stem numbers varied greatly. The divisions were planted in 8-inch glazed pots on August 24, 1946, without any removal of leaf tissue, and were watered when necessary. By September 17 the plants appeared well established and were subjected to soil temperatures of 20°, 26°, and 31° C. by means of the University of Chicago soil temperature tanks. Because of limited facilities only one division of each clone in the unclipped series was subjected to each temperature. For the clipped series, clones 25 and 26 were run singly and clones 4 and 11 in duplicate; they were clipped on October 2. All plants were harvested on November 12, and the dry weights of both roots and tops and the numbers of roots were determined.

Only clones 25 and 4 survived clipping at all soil temperatures. Clone 11 survived clipping at 20° and 26° C., clone 26 only at 20°. The survival of clone 25 and death of clones 26 and 11 at the higher temperatures was possibly related to latitude of origin. This would agree with the observations of NEWELL and KEIM (8) that southern strains were more tolerant of midsummer heat and drought than northern strains. It was quite evident that removal of photosynthetic tissue accompanied by the rapid respiratory rate induced by high root temperatures was very unfavorable for even the strains that survived.

The root systems developed at the lowest temperature were fibrous and much branched, while at the highest temperature few roots of the second and third order were in evidence. STUCKEY (10), working with colonial bent grass, also found maturation of roots to be accelerated by higher soil temperatures, with little branching in evidence.

Total root weight was inversely correlated with soil temperature in all four

clones (table 4), as was the weight of individual roots. The data reflect markedly the limiting effects of high soil temperature upon root growth in this species and also the limiting effect of clipping.

The total numbers of roots of the three clones of northern origin in the unclipped series were also inversely related to soil temperature. However, clone 25, of southern origin, showed the maximum root number at 26° in the unclipped series. In the clipped series the inverse relation to temperature held for all clones except no. 4, where the lowest number of roots was found at 26° C. With the exception of clone 4 at 20° C. and clone 25 at 31°, the numbers of roots were lower on all the clipped plants than on the unclipped plants, irrespective of soil temperature.

Although in general the numbers and dry weights of stems were inversely correlated with soil temperature (table 4), the effects were not so marked as in the roots. Since the underground parts of the stems were subjected directly to the different temperatures, the results do not differentiate the direct from the indirect effects of soil temperature upon the stems.

With the exception of clone 4 at 20° the number of stems of all clones decreased as an effect of clipping, with greater decrease at higher soil temperatures. In the unclipped series one northern clone (no. 11) decreased in live stem number at the highest soil temperature; and the percentage increase in the others was least at the highest temperature. In the southern clone (no. 25) the greatest increase was at 26°.

Total top weight in the unclipped series was inversely correlated with soil temperature in two of the northern clones (26 and 11). Clone 4 yielded most at 20° C., with a slightly greater yield at

31° than at 26°. The southern clone (no. 25) produced the greatest top weight at 26° and the least at 31° C.

In the clipped series the data do not include the weights of tops clipped on October 2. In all clipped plants the final yields at 20° C. exceeded those at 26° or 31°, although in clone 26 the yield at 26° was near that at 20°.

The weight per stem in the unclipped series was inversely correlated with soil temperature for clones 26 and 11 but directly so for clone 4. Clone 25 had the greatest weight per stem at 26° C. and the least at 20°. The surviving plants in the clipped series had the lightest stems at 20° C. with the exception of clone 4 (table 4). This tendency toward fewer but heavier stems in the clipped plants at higher soil temperatures could result from an inhibition in either the initiation or elongation of new tillers with the consequent utilization of available nutrients and reserves by the surviving stems.

A measure of the total yield in proportion to the initial size of the clonal divisions was derived by dividing the final dry top weight by the stem numbers on September 17. In the unclipped series this value in two of the northern clones was inversely correlated with temperature, while in clones 25 and 4 it was greatest at 26° C. Clone 4 showed the least yield at 31° C.

In the clipped series all clones produced less dry-weight yield of tops per root at the higher temperatures, except clone 25, in which it was highest at 26° C. In the unclipped series this value was inversely correlated with soil temperature except in clone 4, in which it was highest at 20° C. and lowest at 26°.

The top-weight/root-weight ratio and weight-per-stem/weight-per-root ratio were positively correlated with soil temperature. This trend in these ratios is

TABLE 4
EFFECTS OF SOIL TEMPERATURE AND CLIPPING

Soil TEMPERA- TURE (° C)	CLONE							
	25		26		4		11	
	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped
Dry wt. of roots (gm.)								
20.....	0.83	0.58	1.30	0.21	5.20	2.01	4.25	0.74
26.....	.79	.33	0.53	§	1.94	0.36	1.41	0.57
31.....	0.11	0.05	0.17	§	0.99	0.42	0.43	§
Wt. per root (mg.)								
20.....	9	8	5	2	27	9	19	5
26.....	7	7	2	§	10	4	11	4
31.....	3	1	1	§	6	4	4	§
No. of roots								
20.....	90	77	265	114	195	220	219	145
26.....	113	50	257	§	189	99	134	129
31.....	37	43	150	§	163	118	121	§
Dry wt. of tops (gm.)								
20.....	2.19*	0.85†	3.81*	0.89†	13.73*	4.78†	15.47*	1.90†
26.....	2.46*	.71†	1.74*	§	9.68*	1.26†	7.80*	1.20†
31.....	0.64*	0.36†	0.71*	§	9.80*	1.41†	3.12*	§
Wt. per stem (mg.)								
20.....	104*	57†	93*	81†	270*	150†	430*	130†
26.....	135*	71†	76*	§	320*	130†	340*	150†
31.....	128*	72†	65*	§	360*	180†	170*	§
Relative no. of stems‡								
20.....	150	83	186	79	196	100	133	68
26.....	200	71	121	§	187	50	135	33
31.....	125	45	110	§	104	40	90	§
Dry wt. of tops per root (mg.)								
20.....	24*	11†	14*	8†	70*	22†	71*	13†
26.....	22*	14†	7*	§	51*	13†	58*	9†
31.....	17*	8†	5*	§	60*	12†	26*	§

* Based on total top yield.

† Based on top yield produced after clipping on October 2.

‡ Number of stems on November 12 as percentage of number on September 17.

§ Dead.

TABLE 4—Continued

SOIL TEMPERA- TURE (° C)	CLONE							
	25		26		4		11	
	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped
	Total top wt./total root wt.							
20.....	2.6*	3.3*	2.9*	7.1*	2.6*	5.9*	3.6*	10.8*
26.....	3.1*	7.0*	3.3*	§	5.0*	13.0*	5.5*	15.2*
31.....	5.8*	13.2*	4.2*	§	9.9*	25.3*	7.2*	§
	Wt. per stem/wt. per root							
20.....	11	19	10	22
26.....	19	36	32	32
31.....	43	59	60	47

related primarily to the strong inverse correlation of root weights, both total and individual, with soil temperature. The adverse effect of clipping upon root growth is indicated by a comparison of the top-weight/root-weight ratios in the clipped series with the unclipped series (table 4).

In this experiment soil temperature exerted relatively greater control over root development than it did upon growth of the tops. Under the conditions of this experiment, adequate moisture was supplied to all plants, and the tops probably did not suffer water deficiency because of inadequate root systems, even at the highest soil temperature. Under field conditions, however, such limitation on root growth with the advent of warm soil temperatures accompanied by lack of moisture in the upper layers of soil would not be conducive to the survival of this species in competition with better adapted species, especially if grazed or mowed simultaneously. The lack of vigor observed in the plants clipped during the summer of 1946

in the greenhouse clipping experiment (table 1) was no doubt in part caused by the removal of photosynthetic tissue with consequent reduction in the synthesis of carbohydrate at the same time that the roots were subjected to high daytime soil temperatures bringing about an increased rate of respiration.

SHADING

Three divisions of the same twenty-three clones used in the greenhouse clipping experiment were used in an experiment on shade tolerance set up at the same time. The plants grew in the full natural light of the greenhouse until June 30, 1946, and were then apparently well established. They were then clipped back to a height of 2 inches but were not clipped thereafter. They were watered as necessary, and each plant was given 192 ml. of nitrate solution on May 4, 1946, on September 5, 1946, and on April 3, 1947. Series C remained in the full greenhouse light; series 1 was shaded by a single thickness of unbleached sheeting of a type used for shade-grown tobacco,

and series 2 by a double thickness. The average values of approximately simultaneous light readings made with a Weston Sunlight Meter during the course of this experiment were: outside, 5233 foot-candles; greenhouse, 2417 f.-c.; under one thickness of sheeting, 731 f.-c.; under two thicknesses of sheeting, 251 f.-c., or a ratio of approximately 100:46:14:5.

By September 27 only four clones had survived in series 2. At the termination of the experiment on June 2, 1947, all series 2 plants had died, but ten clones were still surviving in series 1. The clones which survived longest under the double thickness of cloth were among those surviving under the single thickness almost 8 months later. Six of the plants in series C had died by the termination of the experiment. Two of these were clones that survived in series 1, though not in series 2, and the other four died at about the same time as they did in series 1. Although the light intensity in the greenhouse was less than half that in full sunlight, some factor or factors other than low light intensity must have been the cause of death in most series C plants, as all but one died during the summer and fall of 1946. The same conditions of high air temperatures and infestation with greenhouse pests enumerated in the greenhouse clipping experiment prevailed during the course of this experiment and made growing conditions for all series decidedly adverse. The initial clipping and consequent limitation of photosynthetic tissue in all series, coupled with high air and soil temperatures and lowered light intensity, were quite favorable for a high rate of respiration and limited production of photosynthate.

WATKINS (11) found in a field experiment with *B. inermis* that a reduction of light intensity to 300-800 foot-candles

brought about a decrease in the number of shoots, number of rhizomes, number of fertile shoots, and dry weight of all plant parts, and an increase in the number of elongated internodes and in height of the plant. The effects of shading in the present experiment suggest inherent differences in the shade tolerance of the different clones. There seemed to be little correlation of shade tolerance with latitude of origin, since one of the surviving clones in series 2 had its origin in Washington, Kansas, and one in Saskatchewan. Neither did there seem to be any correlation with growth habit, as clones that were characteristically creepers as well as those that were bunch types were found among both the surviving and the dead plants in all series.

Under field conditions, where the plants grow close together and competition for light as well as for mineral nutrients and water is keen, those individuals that were not tolerant of shading would most certainly be crowded out. Ability to withstand shading, although of selection value in a field of pure brome grass, would be even more important in mixed fields of brome and alfalfa in which the presence of shade-tolerant strains might well determine the survival of brome grass in the mixture.

SOIL VOLUME

Equal divisions of four different clones which had been growing in the garden were planted in containers of seven different types and sizes on August 29, 1946, in an attempt to find what effect soil volume might have upon vegetative growth under greenhouse conditions. Tops and roots were trimmed as little as possible in transplanting, and all conditions under which the plants were grown were similar except the volume of soil in the different containers, the shape of the

containers, and the material of which the containers were constructed (table 5). Although each plant was given water as seemed to be necessary, rapid evaporation through the walls of the smaller unglazed clay pots probably removed the available water from the outer part of the soil mass so rapidly that the plants in these containers did not have sufficient moisture available to them at all times

over the surface of the soil, with some emerging from the space between the soil and the edge of the container. Root distribution was somewhat less uniform and was different for different clones, though in general the roots were well distributed throughout the soil in the larger containers and tended to be concentrated around the bottom and sides of the smaller ones. The roots of all clones in the

TABLE 5
RESULTS IN SOIL-VOLUME EXPERIMENT

Container	Relative soil volume	Original stem count	Final stem count	Final stem count as % of original	Total top wt. (gm.)	Top yield per original stem (gm.)	Av. wt. of each stem (gm.)	Total root no.	Total root wt. (gm.)	Av. wt. of each root (mg.)	T/R	Roots per stem	Top yield per root (mg.)	Wt. per stem/ wt. per root
12-in. square wooden box.	12	22	150	682	35.13	1.6	0.23	754	5.42	7.2	6.5	5.0	4.7	32
12-in., deep clay pot*	10	26	139	534	38.92	1.5	.28	795	8.98	11.3	4.3	5.7	4.9	25
8-inch, glazed crock†	4	28	133	475	38.56	1.4	.29	880	8.15	9.3	4.7	6.6	4.4	31
10-in., deep clay pot*	5	26	114	439	28.93	1.1	.25	590	8.11	13.7	3.6	5.2	4.9	18
10-in., shallow clay pot*	2	27	85	315	13.66	0.51	.16	457	3.74	8.2	3.7	5.4	3.0	20
8-in., deep clay pot*	2	26	79	304	18.98	0.73	.24	491	7.42	15.1	2.6	6.2	3.9	16
6-in., deep clay pot*	1	25	77	308	14.73	0.59	0.19	421	5.68	13.5	2.6	5.5	3.5	14

* Standard greenhouse pots, sides tapering to base.

† 10 inches deep, sides parallel.

for maximum growth. Nitrate solution was applied to all plants on October 15 and November 27 at the rate of 100 ml. per unit of soil volume (volume of 6-inch clay pot taken as unity). Since the applications had no apparent effect upon the vigor of the plants, nitrogen deficiency which could be so corrected was probably not a limiting factor in any container. The plants were harvested on December 10, 1946, and data were obtained on the oven-dry weights of tops and roots and on root numbers (table 5).

In all containers the emergent stems at final harvest were scattered at random

8-inch glazed pots were well distributed through the soil.

The results in table 5 are an average of the values for the four clones at final harvest. With the exception of the 8-inch glazed container, in which results were similar to those in the 12-inch unglazed clay pot, the columns express the results in order of relative volume of container. The final stem count was positively correlated with soil volume as was the percentage increase in stem number. The dry top weight was much lower in the smaller soil volumes, but the trend was not so regular as with stem numbers.

The top yield per original stem was also somewhat proportional to soil volume, although it was least in the 10-inch shallow clay pot. The weight per final stem did not vary so significantly, though it was highest in the 8-inch glazed pot and lowest in the 10-inch shallow clay pot.

Total root number showed no regular relation to soil volume but was greater in the larger containers and was largest in the 8-inch glazed pot. The second largest total root weight was also produced in the 8-inch glazed pot, which had a relatively low soil volume. Dry root weight in general showed no correlation with soil volume, nor did the weight of each root, but the top-weight/root-weight ratio in general was somewhat proportional to soil volume because of the correlation of top weights with the latter.

The number of roots per stem was fairly constant, regardless of the size of the container. The top yield per root and the weight-per-stem/weight-per-root ratio trended with soil volume.

In summary, in contrast to the relative lack of correlation between root data and soil volume, the tops of the plants showed a direct correlation of total stem numbers and total weight with soil volume (table 5). This positive correlation is also primarily responsible for the direct correlation of soil volume with the top-weight/root-weight ratio, the weight-per-stem/weight-per-root ratio, the top yield per root, and the top yield per original stem.

In all the unglazed clay containers the top growth was in direct proportion to the size of container, but in the glazed crock the top growth far exceeded that attained in a slightly larger unglazed clay pot and was almost equivalent to that attained in a clay pot two and a half times as large. It would thus seem advisable to use glazed pots wherever pos-

sible in preference to unglazed clay, and, if unglazed clay pots must be employed, to use the largest ones possible. The limitation placed upon top growth by the 8-inch clay pots in this experiment would seem to indicate that container size was a limiting factor in both the greenhouse clipping experiment and the shading experiment.

Discussion

Based upon the results of these experiments, certain general recommendations can be made with respect to the culture of *B. inermis* under greenhouse conditions. Since high soil temperatures had very adverse effects upon root growth, especially when photosynthesis was curtailed either by removal of leaf tissue or by shading, it would seem advisable to use large pots and to surround them with sand, peat moss, or some other insulating substance to reduce to a minimum the heating effect upon the soil of the extreme daytime air temperatures reached in summer. The results with containers of different kinds indicated that pot size, shape, and type might be an unforeseen limiting factor in many greenhouse experiments. A deep glazed pot of the largest size that is practicable with available space should be used. When it is necessary to carry greenhouse plants that have been weakened by experimental treatment through natural low light intensities comparable to those in winter in Chicago, the use of supplementary artificial illumination during the normal photoperiod would seem advisable.

However, it should be pointed out that, despite the relatively poor growing conditions in the greenhouse, the effects of two, one, and no clippings upon plants in the greenhouse, as measured by the percentage differences in top weights and stem counts in the three series, were

closely comparable to the effects of similar treatments upon plants in the garden, even though absolute values for these criteria of growth were widely different between greenhouse and garden plants.

Whereas a range of soil temperatures and clipping intensities showed stronger correlation with root growth than with top growth, soil volume exerted its effects most markedly upon the tops.

Summary

1. Vegetative responses of diverse plants of smooth brome grass (*Bromus inermis* Leyss.) to variations in soil temperature, container size, clipping intensity, and shading were investigated in the greenhouses and garden of the University of Chicago with plants derived from both clonal divisions and seed from diverse places of origin. The responses of the individual plants were measured in one or more of the following ways: stem count; total oven-dry top weight; root count; and total oven-dry root weight.

2. The effects of clipping upon plants of diverse origins were studied in three separate experiments: clipping in greenhouse (seventeen clones); clipping in garden (twenty-two clones); and clipping seedlings in greenhouse (three strains of diverse origin). Three series were run in the experiments on clones started in April, 1946: series C, control; series 1, clipped once; and series 2, clipped twice in the 1946 growing season. Throughout the course of the experiments with clonal materials, which were terminated in June, 1947, stem counts were made and dry weights of clipped tops determined. Dry weights of tops and roots were determined for the seedlings. At the final harvest dry weights of tops and roots and the number of stems were inversely proportional to frequency of clipping. In the

greenhouse experiment, where light and temperature as well as container size were limiting factors, the weight per stem did not seem to be affected by clipping, but in the garden experiment it was inversely correlated with frequency of clipping. It was further found that clipping was more adverse in its effects upon root growth than upon top growth and that the effect upon both carried over into the season following clipping.

3. Divisions of four clones were grown at soil temperatures of 20°, 26°, and 31° C. Greatest total top weight was produced at 20° C. in three clones of northern origin and at 26° in one clone of southern origin. At all temperatures the increase in number of stems was less in the clipped series than in the unclipped, and the increase was inversely proportional to the soil temperature. One northern clone did not survive clipping at 31° C., and another died at both the 31° and the 26° soil temperatures. The weight of single roots and the total root weight in each clone showed a much stronger inverse correlation with soil temperature than did total top weight.

4. Three divisions of twenty-three different clones were brought into the greenhouse from the garden and planted in 8-inch clay pots in late April, 1946. One series was placed on a well-lighted bench in the greenhouse, another under a single layer of sheeting, and the third was placed under a double layer of sheeting. Relative light intensities were: outside, 100; greenhouse, 46; one layer of cloth, 14; two layers, 5. At the lowest intensity only four clones survived until the fall of 1946. In the spring of 1947, when the experiment was terminated, ten clones were still alive under the single layer of sheeting, seventeen were still alive in the control series, but none had survived in the most shaded series.

5. Divisions of four clones were grown in unglazed clay pots of five different sizes, in 8-inch glazed pots, and in 12-inch-square wooden boxes. With the exception of the plants in the glazed pots, the final stem count, oven-dry top yield, top yield per original stem, total root number, top-weight/root-weight ratio, top yield per root, and ratio of weight per stem/weight per root were directly proportional to the size of the container. In the glazed pots the plants were as vigor-

ous as in clay pots two and a half times larger.

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FLOWERING OF SMOOTH BROME GRASS UNDER CERTAIN ENVIRONMENTAL CONDITIONS¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 588

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Introduction

The increasing importance of smooth brome grass (*Bromus inermis* Leyss.) as a forage crop and in reseeding denuded ranges has led to the necessity for more accurate selection of types adapted to a wide range of latitudes and climatic conditions and to a program of developing improved strains. A knowledge of the conditions necessary for vigorous production of panicles and the setting and maturing of seed, both in the field and under greenhouse conditions, is needed for the efficient carrying-out of such work. In this study a series of clones of brome grass, representing various strains, was examined with regard to these requirements, with especial attention to the production of viable seed, and to determine to some extent the factors involved in the initiation of floral primordia and their subsequent development. The experiments were carried on at the University of Chicago during 1946 and 1947.

The wide range of variation within the species has been recognized for many years. WALDRON (13), in a study of several thousand seedlings of brome grass in North Dakota, found much variation in physiological and morphological characters. He noted a range from a strongly "fertilclinous" condition to a strongly "sterilclinous" one and a similar range in tillering habits. NEWELL and KEIM (7) have reviewed the history of the species

in the United States. In field trials at Lincoln, Nebraska, they noted distinct differences between plants originating from northern sources and those from Nebraska and Kansas in vegetative growth and panicle production. Wide differences among strains in their adaptation to this region were apparent. EVANS and WILSIE (3), in greenhouse experiments, found that three clones—early, midseason, and late-maturing, respectively—responded differently to changes in daylength, temperature, and level of fertility. WATKINS (14), using seed of a commercial strain from Saskatchewan, concluded that brome grass was an indeterminate species photoperiodically, in the sense of GARNER (5), in that most fertile shoots were produced on natural daylength. ALLARD and EVANS (1), however, after finding no flowering on photoperiods of 10–13 hours and more vigorous flowering with longer photoperiods beyond 13 hours, assigned the species to the long-day class.

Material and methods

To insure genetic uniformity clonal material was used in all experiments. The clones available at the time of experimentation provided a range of morphological habit and place of origin.² OM-1 traces back to a plant selected for selfing in a bulk lot of the Canadian variety, Parkland. OM-2 goes back to a Nebraska

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² Material of all clones and data on their origin were supplied by Dr. ETLAR L. NIELSEN, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture.

strain, B-4. OM-3 is a selfed line out of PI 115331 from Leningrad, Russia. Cl 3 represents the isolation of the better plants from PI 115334 from Leningrad. Cl 8 is an isolation from FC 22435 (original source not known at the time of writing). Cl 9 is an isolation from PI 101647, received from Manchuria. The origin of Cl 19 and 65 is not known. Cl 23, 27, 31, and 84 are all selected plants from bulk lots of Parkland seed. 914-1 is from the first inbred generation of a plant of the Parkland variety. The plants used represent the species over a latitudinal range of approximately 20°.

Equal divisions of each clone were made from plants previously established in the experimental garden and were planted in 8-inch unglazed clay pots. The soil used was a heavy clay loam of prairie origin, lightened with a mixture of sand and fine gravel. It undoubtedly contained a supply of mineral nutrients adequate to prevent deficiencies during the course of the experiments. Ten days after potting, all plants had shown vigorous growth. They were watered as often as was necessary to keep the soil constantly moist.

Supplementary light in experiments I and II was supplied for all except two series by banks of twelve 40-watt fluorescent lamps of the "Daylight" type mounted $2\frac{1}{2}$ inches apart on a white-enameled reflecting board. These gave a light intensity of about 800 foot-candles at a distance of 10 inches below. Lights were raised periodically as the plants grew. Pots containing plants of slower growth were elevated individually to keep the general foliage level of a series fairly uniform.

In experiment I in 1946, two of the series, designated 17M and 15M, were given supplementary light from 200-watt incandescent-filament lamps mounted in

individual reflectors. Light intensity at plant level was about 150 foot-candles. These two series were placed on movable trucks and received natural daylight for 9 hours in the open greenhouse and artificial light for 8 and 6 hours, respectively, after being moved into ventilated light-proof sheds.

Three of the series in 1946 were grown on a greenhouse bench with the banks of fluorescent lamps suspended above them, and the lights were kept burning throughout the light period. Black cloths, coated white on the inside surface, were hung from each reflector to inclose the plants during the dark period. These were opened on the side to the south during the day, and on sunny days the reflectors and cloths were raised to allow maximum and equal illumination of all plants. Temperatures inside the cloths were found to vary considerably, long photoperiods resulting in higher temperatures; consequently, later experiments were run in the sheds or in the open greenhouse without the inclosing cloths.

Flowering response was evaluated by noting the first exertion of the inflorescence from the ensheathing leaves and by the later development and vigor of panicles. In some cases the status of the apical meristem in respect to vegetative or reproductive condition was determined by dissection and microscopic examination. Vegetative response was evaluated mainly by maximum height of plants, number of new tillers produced, number of tillers showing elongation, and dry weights of roots and tops at final harvest.

Experimentation

EXPERIMENT I.—Twelve dormant plants were brought into the greenhouse on January 10, 1946, and on January 11 small pieces of rhizomes, fairly uniform

as to size and condition of growing points, were planted in individual pots. They rapidly resumed growth, and on January 22 one pot of each clone was placed under each of six treatments. Three photoperiods—17, 15, and 13 hours—were used with fluorescent light, and two photoperiods—17 and 15 hours—with incandescent-filament light. A series (*N*) was also run on natural daylength and light intensity.

Air temperatures given in table 1 represent averages of measurements over the course of the experiment, which was

TABLE 1
AIR TEMPERATURE (IN ° F.) AT PLANT
LEVEL. EXPERIMENT I

Series	Av. min.	Av. max.	Range	Mean
17-hour.....	68.6	85.3	69-90	77.0
15.....	66.5	84.8	62-89	75.7
13.....	66.2	82.3	62-90	74.3
17M.....	63.3	75.2	55-79	69.3
15M.....	58.3	69.8	51-74	64.1
N.....	63.7	70.8	58-90	67.3

terminated, except for the *N* series, on April 18, 1946.

Flowering response, as based on the date on which the first inflorescence was exerted in each plant, is shown in table 2. Anthesis followed in approximately 14 days in all cases, with the exception of the weak or sterile panicles. Six of the twelve clones flowered on 17-hour photoperiod; four of these six flowered also on 15-hour photoperiod, though less vigorously and from 5 to 26 days later. Five of the six flowered also on 13-hour photoperiod, in addition to two clones that flowered only on this light period. Inflorescences on 13-hour plants were at best of only fair vigor in comparison with 17-hour plants. Three of the controls on natural daylength flowered, though poorly and from 34 to 44 days later than on 17-hour photoperiod.

Differences in vigor of growth and flowering between the 17-hour series under fluorescent and under incandescent-filament light were marked. Five plants of the latter series flowered, producing rather poor panicles from 4 to 21 days later than the plants of the same clones under the higher light intensity of the fluorescent lamps. The 15M series was inferior in all respects to any of the

TABLE 2
NUMBER OF DAYS UNTIL FLOWERING AFTER
BEGINNING OF PHOTOPERIODIC
TREATMENTS ON JANUARY 22

CLONE	SERIES					
	17	15	13	N	17M	15M
OM- 1.....	68
2.....
3.....	23	28	37*	33*	47*
Cl 3.....	24	30	68*	28	46*
8.....
9.....	17	33	38
19.....	28	54	38
23.....	56*
27.....	22	48*	38*	56*	28*	46*
31.....
65.....	21	28	28*	56*
84.....

* Poor and sterile panicle.

others. Three plants exerted inflorescences in 46 days, but they were of insufficient vigor to reach anthesis. It is emphasized that the 17M and 15M series received only natural daylight for 9 hours of the photoperiod, while the series under fluorescent light received artificial light continuously during the photoperiod. Considering the many very cloudy days of the Chicago winter as well as the relatively low intensity of the supplementary incandescent-filament light, the total illumination of the M series was undoubtedly below the minimum required for normal growth. The lower range of temperatures (table 1) to which these plants

were exposed during the period of experimentation was probably another factor contributory to their lack of vigor.

No seed was matured by any plants, although the panicles produced by the 17-hour plants under fluorescent light appeared to be vigorous and the pollen under microscopic examination to be normal. It was thought probable that the high temperatures resulting from inclosing the plants and lights with heavy lightproof cloth were responsible for this failure. Further experiments were modified to provide better control of this factor.

In vegetative response there were marked differences among treatments under fluorescent lights. The twelve clones, at the time of harvest on April 18, had produced totals of 74, 56, and 26 elongated tillers on 17-, 15-, and 13-hour photoperiods, respectively. Numbers of unelongated tillers were inversely correlated with photoperiod, with totals of 97, 146, and 218. Total numbers of tillers—171, 202, 244—were also inversely correlated with length of the light period. Maximum height was measured from the soil surface to the tip of the longest leaf or inflorescence and was directly correlated with length of photoperiod for most clones, the averages being 66.6, 55.6, and 50.0 cm., respectively. These differences were clearly reflected in the general aspect of the plants (figs. 1-3).

Comparable data for the 17M and 15M series, respectively, are: elongated tillers, 44 and 15; unelongated tillers, 37 and 121; total tillers, 81 and 136. They show clearly the limited growth made under low light intensity and average low temperature in these series in contrast with those under fluorescent lights, and also the effect of the longer photoperiod in inducing elongation of tillers.

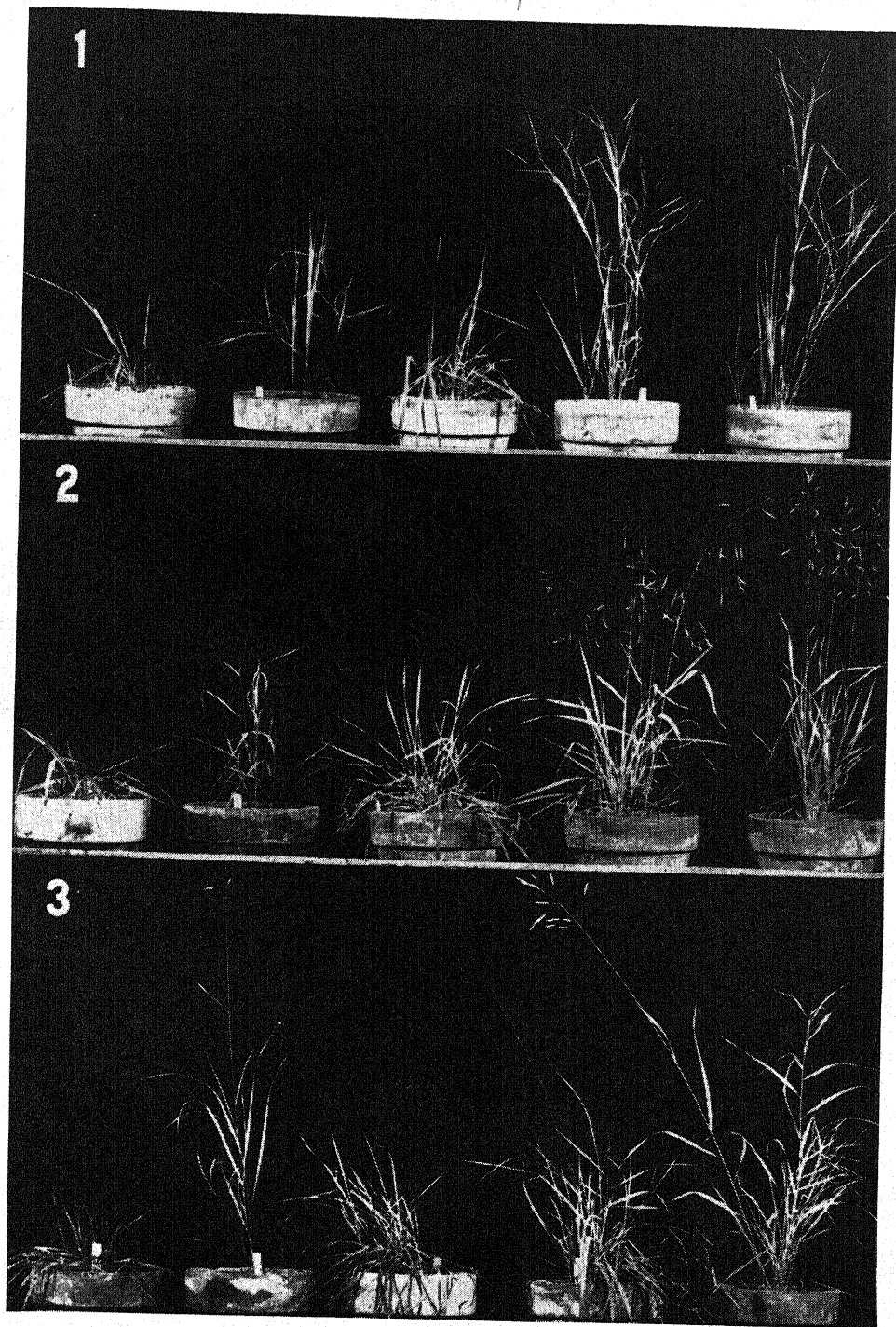
Total numbers of rhizomes for the

twelve clones showed no apparent influence of photoperiod but a strong influence of light intensity and temperature. The 17-, 15-, and 13-hour plants produced a total of 40, 39, and 41 rhizomes, respectively, while the 17M and 15M series produced only 5 and 6.

EXPERIMENT II.—In this experiment plants were brought into the greenhouse from the garden at two different times, on August 31, 1946, and again on December 27, to test the effect of exposure to low temperatures and of long establishment in the pots on floral initiation and emergence. Six of the clones used in experiment I were used. Of these, Cl 9 and Cl 27 had flowered readily on long photoperiod, OM-1 and Cl 23 had flowered only on a short light period, and OM-2 and Cl 84 had not flowered on any treatment (table 2). Four divisions of each were made on each date. Large pieces of entire plants were used for each division rather than single rhizomes.

After potting, the August plants were trimmed of dead parts and old sterile culms but otherwise left intact. They were kept on natural daylength in the greenhouse until differential treatment was begun on January 7, 1947. During this period they were given several hours of artificial light on very cloudy days to prevent too great loss of vigor; this was supplied without extending the photoperiod. Growth during September to January was limited, and most of the plants had declined perceptibly in vigor by the time treatments were begun. Food reserves for further growth were thus probably low, and the response of these plants to photoperiodic treatments in contrast to that of the December plants may have been conditioned by this fact as well as by lack of pre-chilling.

On January 7, two series, each including August and December plants, were



FIGS. 1-3.—Clonal divisions of smooth brome grass grown from single rhizomes brought into greenhouse on January 10, 1946. After January 21 they were subjected to (*left to right*): Chicago natural daylength and light intensity (series *N*); 17 hours of relatively low light intensity (series 17M); 13, 15, and 17 hours of relatively high light intensity (fluorescent light). Fig. 1, Cl 84; fig. 2, OM-3; fig. 3, Cl 27. (Photographed April 16, 1946.)

placed on movable trucks in lightproof sheds. Banks of twelve 40-watt fluorescent lamps as described above were used to provide 18- and 13-hour photoperiods. On sunny days the trucks were moved out of the sheds to provide maximum light intensity throughout the light period. In addition to a series (*N*) on natural daylength and light intensity in another greenhouse room, an additional 18-hour series (18C) was run under

TABLE 3
AIR TEMPERATURES (IN ° F.) AT PLANT
LEVEL. EXPERIMENT II

Series	Av. min.	Av. max.	Range	Mean
18-hour....	67.0	74.3	63-82	70.7
18C.....	66.8	74.3	57-87	70.6
13.....	63.8	72.6	55-79	68.2
<i>N</i>	66.6	74.1	59-88	70.4

fluorescent lights in still another greenhouse room as a check on conditions in the sheds.

Temperature data (table 3) indicate a much better control of this factor than in 1946.

Flowering data are given in table 4. Of the plants brought in on December 27, all clones flowered on 18-hour photoperiod within 26 days. Anthesis followed about 2 weeks later. There was good agreement between the 18 and 18C series. One clone, Cl 9, flowered on 13-hour photoperiod, 20 days after first flowering on long photoperiod. Series 18, 18C, and 13 were terminated on March 29. None of the control (*N*) plants had flowered by June 1, although the same clones then outdoors had done so, as they also did when brought into the greenhouse on April 14 and left on natural daylength. This contrast in plants differently treated on natural daylength is further evidence that exhaustion of carbohydrate reserves may be a factor involved in failure to flower under greenhouse conditions.

Of the August plants, all on 18-hour photoperiod showed marked tiller elongation (figs. 4-6), but no inflorescences were produced. Stem tips of these plants were examined at the close of the experiment on March 29, and all were found to be vegetative.

Tiller counts were made of all plants on January 23 and again on March 26 (table 5). For the August plants, averages of increase in tiller number in the 2 months for the two 18-hour series were lower for each clone than for the 13-hour series. Tiller counts for the December plants showed less correlation with length of photoperiod. This may perhaps be attributed to the shorter time available to these plants for becoming established in the pots before being placed under the lights. Relatively, the *N* series produced most new tillers and the 13-hour series the least.

TABLE 4
NUMBER OF DAYS UNTIL FLOWERING AFTER BE-
GINNING OF PHOTOPERIODIC TREATMENTS
ON JANUARY 7. DECEMBER PLANTS

CLONES	SERIES			
	18	18C	13	<i>N</i>
OM- 1.....	23	23
2.....	26	26
Cl 9.....	21	20	41
23.....	26	23
27.....	23	23
84.....	22	30

Average maximum heights on March 21 for series 18, 18C, 13, and *N*, respectively, were: August plants, 69, 74, 43, and 34 cm.; December plants, 79, 83, 55, and 38 cm. Height was thus greatest in the 18-hour plants and least in the *N* series. December plants were taller than August plants on 18-hour photoperiod, as in most cases the flowering culms overtopped the vegetative tillers of the latter.

Dry weights of tops (table 6) of the August plants were greatest in the 18-hour series; of roots, in the 13-hour series. The plants were cut off at the soil surface, and all below-ground parts were evaluated together as roots with no attempt to distinguish rhizomes and stem bases.

The December plants showed trends similar to those of the August plants in regard to dry weights. Their absolute weights were somewhat greater; since the initial divisions in August and December were approximately equal, this may reflect the failure of the August plants to grow vigorously after being brought into the greenhouse.

Four other clones that were still available, namely, Cl 8, 19, 31, and 65, and a new plant, 914-1, were also brought in during December and were subjected to somewhat similar treatments as the

light was supplied until it was evident that the panicles were mature. Under these conditions, most of the 18-hour plants which had flowered also formed seed which produced vigorous seedlings when planted.

EXPERIMENT III.—An important point in regard to the flowering of brome grass to be determined was the time of initiation of observable floral primordia in the Chicago area. To ascertain this,

TABLE 6

DRY WEIGHTS (GM.) OF PLANTS HARVESTED
MARCH 29. AVERAGES ONLY

PLANTS	18-HOUR		13-HOUR	
	Tops	Roots	Tops	Roots
August.....	8.83	7.49	6.88	8.97
December.....	10.30	9.19	6.09	9.56

TABLE 5

PERCENTAGE INCREASE IN NUMBER OF TILLERS
PER PLANT, JANUARY 23—MARCH 26

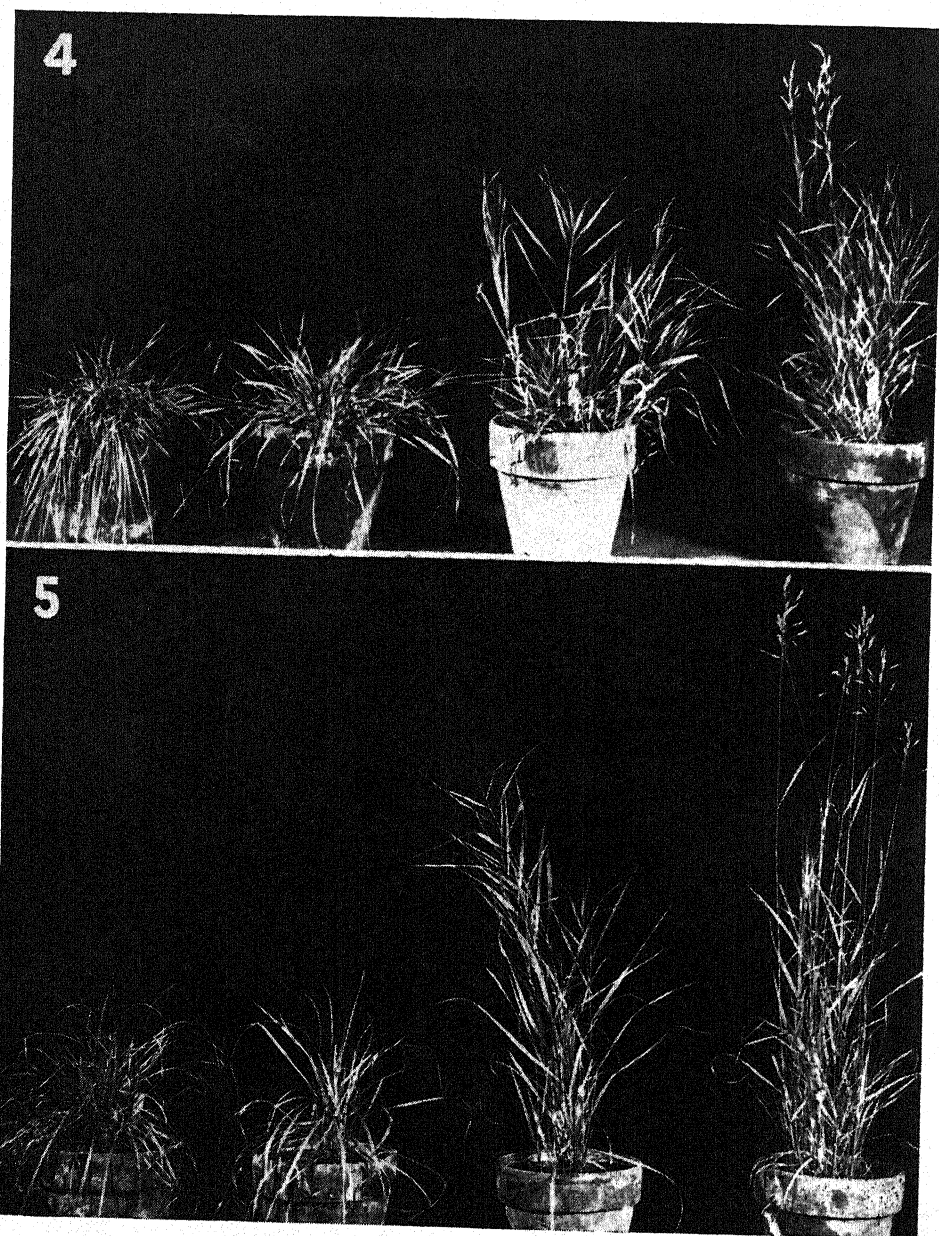
PLANTS	SERIES			
	18	18C	13	N
August.....	13.7	36.2	42.8	77.8
December.....	27.7	38.6	27.1	68.6

other six. Results were substantially in agreement. Clone 914-1 was the only plant of this group to bloom on 13-hour photoperiod after 39 days. It also bloomed on the 18-hour photoperiod after 21 days.

Failure to obtain the production of seed in experiment I was a principal reason for repeating the work in a revised form. In 1947, panicles of different clones were shaken together when in anthesis to insure pollination, since the species has a high degree of self-sterility (2). Artificial

stem tips of plants of the eleven clones still in the garden in 1947 were dissected out periodically during the spring after growth had been resumed. Floral primordia were first discovered on Cl 9 on April 8, on OM-1, Cl 27, Cl 84, and 914-1 on April 9, on Cl 23 on April 10, and on OM-2 on April 14. In all cases the reproductive growing points were located at or near the ground level in unelongated shoots 20 cm. or more in height. The spring of 1947 was abnormally cold, and these dates may be considerably later than the average time of initiation.

To determine to some extent the conditions necessary for the further development and elongation of the inflorescences, plants of these seven clones were brought in on April 14, potted, and put on 13-hour photoperiod, receiving 9 hours of natural daylight in the open greenhouse and 4 hours of supplementary light supplied by three 30-watt fluores-



FIGS. 4, 5.—Clonal divisions of smooth brome grass grown after January 6, 1947, on photoperiods of 13 hours (*left pair*) and 18 hours (*right pair*). Left-hand plant of each pair was brought into greenhouse on August 31, 1946; right-hand plant of each pair, on December 27. Fig. 4, OM-1; fig. 5, OM-2. Initial clonal divisions were much larger than for plants shown in figs. 1-3. (Photographed March 28, 1947.)

cent lamps in the shed. On the basis of the samples dissected, the majority of the larger tillers on all clones were then in the reproductive state. By May 12 clone 914-1 had produced several very depauperate inflorescences, and, by May 18, OM-1, Cl 9, and Cl 84 had produced completely barren elongated culms. On June 3 the remaining plants of this group

that none of the tillers on the section brought in were reproductive on April 14.

These observations are in contrast to the behavior of divisions of clones Cl 23 and 914-1 also brought into the greenhouse on April 14 and allowed to grow under the increasing natural photoperiods. Tillers of these, dissected on April



FIG. 6.—As in figs. 4, 5. Cl 9

on 13-hour photoperiod were examined by dissection. Cl 23 had several tillers with panicles well developed and nearing exsertion, but the branchlets of the panicles were brown and withered except for the lowermost two or three, which were still green. About six internodes had elongated in each tiller examined. Cl 27 was in a similar condition but with inflorescences less developed. OM-2 showed only vegetative stem tips. This was a very small plant because of a lack of material in the garden, and it is possible

23, were found to have inflorescences up to 11 mm. long with branches well developed. One plant flowered on May 2, with normal panicles, and all were in bloom by May 14. This is in sharp contrast to the failure of the *N* series of experiment II to flower on the even longer photoperiods of late May and early June. It is possible that the *N* plants required a longer period of chilling in order to flower, comparable to that received by the plants brought in on April 14. It is more probable, however, that the subjec-

tion of the December *N* series to the adverse effects of 3 months of dark and short but relatively warm winter days in the greenhouse was more significant than the amount of chilling, in view of the excellent flowering of the December plants on 18-hour photoperiod after the same amount of chilling. The failure of the August plants to flower on 18-hour photoperiod may also be attributed partly to the adverse effects of too long a period in the greenhouse in small pots with short photoperiods and low light intensity.

Discussion

The pronounced effect of the longer photoperiods in promoting culm elongation and, under some conditions, the production of panicles in brome grass is in agreement with the results of most other investigators. The differences in the amount of flowering and in the time required for flowering between the plants on long photoperiods and those on natural daylength in winter were marked.

That the effect of long photoperiods is not primarily or perhaps not at all responsible for the initiation of inflorescences is indicated. Attainment of the physiological condition resulting in the formation of floral initials as the first morphological sign of the reproductive state is with little doubt caused by a complex of factors. The interrelations of temperature with photoperiod have been examined for a number of species of plants (10, 15). In the case of sugar beets, it has been found difficult to distinguish the roles of the two factors, and induction of flowers under the influence of prolonged low temperatures and long photoperiods is tentatively regarded as a single process (9). In brome grass the importance of chilling in the process is probably marked in many individuals in the

species, even though in the clonal material used by EVANS and WILSIE (3) some of the plants brought in during October flowered well under certain conditions. The degree of chilling to which they had been subjected was unspecified. The need for chilling for floral initiation has been reported for other species of grasses. Some unspecified American work on cocksfoot is reported by WHYTE (15). Plants grown for 6 months under a 16-hour photoperiod in a greenhouse produced no heads; preconditioning by growing on short photoperiods and low temperature for 2-3 months resulted in flowering after removal to the 16-hour day. WHYTE also reported (15) that a late-flowering strain of perennial ryegrass was induced to flower early after exposure to winter conditions in Wales followed by transfer to continuous light plus higher temperatures.

Pre-chilling, however, is not invariably essential to flowering in brome grass. On July 1, 1946, seven clones were observed in anthesis in the greenhouse, including three used in the present work—Cl 9, 65, and 84. These plants had been in the warm greenhouse since June, 1945. This plant of Cl 9, still in the greenhouse, flowered again in 1947, on June 20. The August plant of the same clone in the *N* series of experiment II also was in flower on June 20. The internal balance or possible specific substances necessary to cause the change from the vegetative to the reproductive state apparently can be reached or formed through more than one complex of causes, in which temperature, light, age of plant, nutrition, genetic factors, and others, may be operative at various levels to produce the same end result.

The fact that floral primordia are formed by early April on brome grass in the field at Chicago indicates that a long

photoperiod is not required for initiation during the season of flowering, whatever may be its role in the previous season. KNOBLOCH (6) observed that on May 1 at Ames, Iowa, inflorescences are found near the ground level and are 1-1.5 cm. long. He stated that panicle primordia are initiated only in the year of anthesis. In northern Ohio the growing points of many perennial grasses remain vegetative during late summer and fall; floral primordia become visible in April or very early in May of the following year (4). WHYTE (15) concluded that it appears in England that "although grasses such as cocksfoot and ryegrass may be ripe-to-flower during the winter months (with no external evidence of this condition), the flower primordia are not laid down until March of the following year."

Long day, then, if it is an essential factor in floral initiation in brome grass, must apparently affect the plant in one growing season and the physiological effect be carried over to the next. It is not difficult to agree with SHARMAN, as quoted by WHYTE (15), who doubted whether daylength is of importance in the change from the vegetative to the reproductive state in perennial grasses such as ryegrass or sweet vernal.

Normal development and elongation of the culms and of the panicle initials, on the other hand, seem, in brome grass, to be definitely related to certain photoperiods. This is evidenced both by its flowering on natural daylength as the season advances and by the experimental results. It is significant that the clones referred to above as flowering without pre-chilling did so only after the days had lengthened, though temperature conditions did not preclude flowering at any time of year. Also, when plants with floral primordia already present were placed on 13-hour photoperiods, exser-

tion of the inflorescences was effectively inhibited in three clones, with only barren or depauperate panicles produced in the four others tested. The degenerating state of the inhibited inflorescences was similar to that described by TINCKER (11), who found that plants of early timothy on 6-, 9-, and 12-hour photoperiods all produced tillers containing young flowers checked in development and dying off. Sweet-scented vernal grass, prevented from flowering by 6-hour photoperiod, had floral primordia consisting of minute outer glumes inclosing a group of meristematic cells. In the Gramineae in general, he concluded, it is possible for the primordia (rachis and glumes) to be laid down and development arrested at such a stage (12).

Two clones of brome grass, OM-1 and Cl 23, flowered only on short photoperiod in experiment I and only on long photoperiod in experiment II. Since, however, they flowered 45 and 30 days earlier, respectively, on long photoperiod than on short, it is reasonable to assume that the longer photoperiod is more favorable for the completion of reproductive activity. The fact that both OM-2 and Cl 84 flowered on 18-hour photoperiod in 1947 and failed to flower on a 17-hour photoperiod in 1946 could be interpreted to mean that the lower critical limit for flowering of these clones lies between 17 and 18 hours. However, it is perhaps more reasonable to believe that the large pieces of clonal material used in 1947 were more representative of the state of the entire plant at the time of bringing it in than were the single pieces of rhizome used in 1946. Growing-points of the segments used in 1946 may not have been "ripe-to-flower" because of age, position on the plant, or other factor.

Vegetative behavior under the conditions of these experiments was consistent

with that reported by others for brome grass (1, 3, 14) and for other species of grasses (1, 8, 11, 15). Plants on short photoperiods tillered more abundantly than those on long photoperiods and showed little stem elongation. Shoots produced on short photoperiod were generally decumbent or semi-decumbent, resulting in a rosette type of plant. On 18-hour photoperiod marked elongation of sterile shoots occurred in the August plants of experiment II, so that at harvest the plants closely resembled the types called "sterilclinous" by WALDRON (13). This also occurred in the 18-hour December plants in two or three instances (fig. 6) in which sterile shoots continued growth after maturation of the panicles on other culms and finally overtopped the flowering stems.

Growth and flowering responses of brome grass in these experiments showed no apparent correlation with place of origin of the clones. Differences of a few days in time of flowering cannot be held significant when the approximate nature of the data is considered. The thirteen clones behaved in a somewhat similar manner physiologically under the experimental conditions, in spite of their diversity in origin and habit, with the possible exception of the Manchurian clone, Cl 9. This plant was consistently the first to flower on long photoperiods and bloomed also on 15- and 13-hour light periods. It has flowered as well without preconditioning by chilling. Vegetatively it was the tallest of the clones and produced the greatest weight of both tops and roots.

It is apparent that a combination of conditions is necessary to insure most vigorous growth, flowering, and production of seed of brome grass in the greenhouse in winter. In these experiments these ends were accomplished most successfully when the plants were brought

in from the field after a period of chilling and were allowed to grow in the pots for only 10 days before being subjected to a relatively high intensity of supplementary light and long photoperiods. Fairly large pieces of transplant material were more successful than single rhizomes.

Summary

1. Flowering and growth responses of thirteen diverse clones of smooth brome grass were studied in greenhouse experiments, supplemented by observations made on plants established in the field, conducted during the winter and spring of 1946 and 1947.

2. In early 1946, clonal divisions, consisting of single sections of rhizomes, were grown on Chicago natural daylength and light intensity and on 17-, 15-, and 13-hour photoperiods under fluorescent lamps providing relatively high light intensity and under incandescent-filament lamps providing relatively low light intensity. Flowering first occurred approximately 1 month after bringing the plants in from the field in early January. Earliest and most prolific flowering and most vigorous vegetative growth occurred on 17-hour photoperiod under fluorescent light. The number of elongated tillers was directly, and of unelongated tillers inversely, correlated with length of photoperiod. Rhizome production was not obviously affected by photoperiod but was strongly affected by light intensity.

3. Plants were brought in from the field on August 31, 1946, and again on December 27 after exposure to low temperatures. Clonal divisions of both groups of plants, consisting of pieces of entire plants, were grown on 18- and 13-hour photoperiods under fluorescent lamps, beginning January 7, 1947, and on natural daylength. Flowering of the 18-hour De-

cember plants occurred in approximately 3-4 weeks after differential photoperiodic treatments were begun. One December plant on 13-hour photoperiod flowered. No December plants on natural daylength had flowered by June 1. None of the August plants flowered under any treatment, but all on long photoperiod showed marked tiller elongation. Increase in number of tillers was greatest for the August plants on natural daylength, least for those on 18-hour photoperiod. Of the December plants, the series on natural daylength produced most new tillers, plants on 13-hour photoperiod least. Dry weights of tops of both August and December plants were greatest in the 18-hour series, of roots in the 13-hour series. Height in both August and December plants was greatest in the 18-hour series, least in the series on natu-

ral daylength. Fully viable seed was produced on the 18-hour December plants under the relatively poor growing conditions of a Chicago winter in the greenhouse.

4. Floral primordia were present on plants growing in the field at Chicago in early April. A 13-hour photoperiod inhibited their subsequent normal development and elongation.

5. The vegetative and flowering responses showed no apparent correlation with place of origin of the clones.

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REACTION OF CERTAIN PLANT GROWTH-REGULATORS WITH ION EXCHANGERS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 589

ROBERT J. WEAVER

Introduction

Several investigators have reported that 2,4-dichlorophenoxyacetic acid (2,4-D) and certain other plant growth-regulators are readily leached from soils (2, 3, 7). NUTMAN *et al.* (7) found that leaching equivalent to 1.4 inches of water resulted in a loss of 2,4-D from the soil but that far greater volumes of water did not completely remove it. This would indicate that some 2,4-D may be adsorbed by the soil. HANKS (3) studied rates of leaching of 2,4-D and its calcium salt from six types of soil and found that the two compounds were leached from any one soil at about equal rates, as shown by comparative toxicities of the leachates, but were differentially leached from the various soils. It was recently shown by LUCAS and HAMNER (5) that 2,4-D is inactivated by adsorption on charcoal. Bean plants sprayed with a 0.1% solution of sodium 2,4-dichlorophenoxyacetate, which had been mixed and shaken with 1% activated charcoal, showed little injury.

The degree of adsorption of several growth-regulators by certain ion-exchange materials and also the readiness with which the compounds are eluted after having been adsorbed are reported here. Growth of plants in exchanger materials containing an adsorbed growth-regulator was also observed. Such studies may help to explain why herbicidal growth-regulators vary in toxicity in different soils and why the rates of leaching of the compounds from different soils may vary. A spectrophotometric method

developed by BANDURSKI (1) for determining growth-regulators in solution made possible quantitative studies of adsorption and leaching of such compounds which could heretofore be done only with difficulty when using bioassays (6, 8).

Material and methods

The cation exchangers used were a resin exchanger (Amberlite IR-100), a carbonaceous exchanger (Zeo-Karb H), a synthetic sodium aluminosilicate (Decalso), and a processed glauconite (Zeo-Dur). The anion exchangers were amine resins (Amberlite IR-4B and De-Acidite). The Amberlites were obtained from the Resinous Products and Chemical Company, and other exchangers from the Permutit Company. Hereafter the word "Amberlite" is omitted when this type of exchanger is mentioned.

All cation exchangers were screened so that the size of the particles was smaller than 20 mesh but larger than 40 mesh. IR-100 and Zeo-Karb H were stirred with 5% hydrochloric acid, which was changed several times, for a period of 48 hours. Decalso, Zeo-Dur, and IR-100 were treated with several changes of 4% sodium chloride or calcium chloride solution over a period of 24 hours. The materials were then washed thoroughly with distilled water until free of hydrochloric acid or the chloride salts. They were dried in an oven at 40° C. and then placed in tightly stoppered bottles.

The anion exchangers were prepared by stirring with several changes of 5%

sodium carbonate solution over a 24-hour period. The materials were then dried for about 5 hours at 40° C. and placed in tightly stoppered bottles.

Moisture contents of the prepared materials were determined by heating $\frac{1}{2}$ -gm. samples in an oven at 100° C. (table 1) to make possible the calculation of the capacities of the various exchangers on a comparative basis.

Six plant growth-regulators were used for experimentation: 2,4-dichlorophenoxyacetic acid (2,4-D), ammonium 2,4-dichlorophenoxyacetate (NH_4 2,4-D), cu-

the complexes as established with the spectrophotometer.

The spectrophotometric method developed by BANDURSKI (1) was employed to measure the compounds in solution. A quartz spectrophotometer, Beckman model DU, using a hydrogen discharge tube as the light source and silica sample cells, was employed. The absorption maximum of 2,4,5-T in water was located at 2,880 Å and that of IPPC at 2,690 Å. The absorption maximum of 2,835 Å was used for 2,4-D in water (1).

PROCEDURE FOR STATIC TRIALS

Static trials were divided into two types: (a) those in which it was determined how much of a compound an exchanger material removed from a solution and (b) those in which elution of regulators from exchangers was studied.

In static trials 0.5-gm. samples of the exchanger were placed in 250-ml. Erlenmeyer flasks. Fifty milliliters of the appropriate solution were pipetted into the flask, which was then stoppered. The flask was allowed to stand for 48 hours with frequent shaking. At this time the concentration of the growth-regulator in the supernatant liquid was determined, and the amount of compound adsorbed or eluted was calculated. Each test was run in duplicate or triplicate.

In elution studies the compounds were usually added to the exchangers by dissolving the growth-regulators in 95% ethyl alcohol and shaking with the exchangers for about 30 minutes. The alcohol was then evaporated by placing the container in a circulating oven at about 70° C. The growth-regulators were also added to exchanger materials by shaking the exchangers with aqueous solutions of the compounds. The amount of regulator adsorbed by the exchanger was calculated by measuring the decrease in con-

TABLE 1
MOISTURE CONTENT OF EXCHANGE MATERIALS

Exchange material	Cycle	% moisture
Amberlite IR-100 H.....	H ⁺	12.4
Amberlite IR-100 Na.....	Na ⁺	5.6
Amberlite IR-100 Ca.....	Ca ⁺⁺	6.0
Zeo-Karb H.....	H ⁺	5.8
Decalco.....	Na ⁺	7.6
Zeo-Dur.....	Na ⁺	7.6
Amberlite IR-4B.....	Anion	10.6
De-Acidite.....	Anion	36.6

pric 2,4-dichlorophenoxyacetate [$\text{Cu}(2,4\text{-D})_2$], calcium 2,4-dichlorophenoxyacetate [$\text{Ca}(2,4\text{-D})_2$], 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and isopropyl N-phenylcarbamate (IPPC). The 2,4-D was purified by running it through several salt-acid cycles. $\text{Cu}(2,4\text{-D})_2$ was prepared by causing an excess of an aqueous solution of NH_4 2,4-D to react with a solution of cupric chloride, and then washing the precipitate of $\text{Cu}(2,4\text{-D})_2$ free of ammonium chloride. $\text{Ca}(2,4\text{-D})_2$ was made by adding calcium chloride to an aqueous solution of NH_4 2,4-D. The 2,4,5-T and the IPPC used were from commercial sources and were assumed to be relatively pure. The purity of 2,4-D and its salts was confirmed by observation of the molecular extinction values of

centration of the compound in the supernatant liquid.

PROCEDURE FOR LEACHING EXPERIMENTS

Pyrex-glass tubes about 40 mm. in diameter and 25 cm. in height were used for studies of leaching (fig. 1). The tubes were tapered at the lower end so that the bottom 5 cm. were about $\frac{1}{4}$ inch in diameter. The exchanger bed in each tube, about 10 cm. in height, was supported by a plug of glass wool at the point of constriction in the tube. A piece of rubber

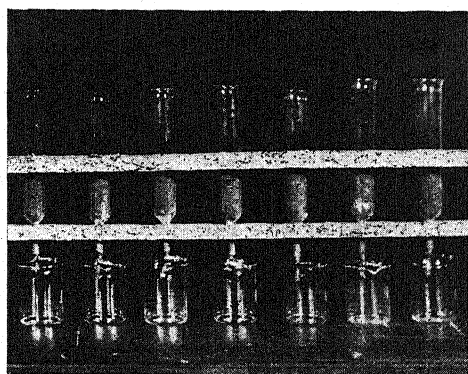


FIG. 1.—Equipment for leaching experiments

tubing about $1\frac{1}{2}$ inches long and with a screw-type clamp was fitted over the lower end of the leaching tube. A reservoir of liquid could thus be maintained above the exchanger material.

After the exchanger material was placed in the tube, distilled water was slowly forced into the lower end of the tube until the level of water reached the top of the exchanger bed; the screw clamp was then closed. This backwashing was necessary in order to prevent presence of air pockets in the bed. After allowing to stand for 15 minutes, 55 ml. (a 2-inch column) of distilled water was added above the bed. The screw clamp was then opened so that a slow drip was obtained which allowed 55 ml. of water

to leach through in about 45 minutes, after which the clamp was closed. During leaching the tubes were frequently rotated so that the flow rate would be uniform throughout the bed. All tests were run in triplicate.

Experimentation

The objective of one experiment was to determine the relative adsorptive capacities of six exchanger materials for six growth-regulators. Aqueous solutions of 0.000226 normality of 2,4-D (50 p.p.m.), $\text{NH}_4\text{2,4-D}$, Cu(2,4-D)_2 , Ca(2,4-D)_2 , 2,4,5-T, and IPPC were prepared. IPPC was considered to have a valence of one. Fifty milliliters of these solutions were added to 0.5-gm. samples of air-dry IR-100 H, Zeo-Karb H, Decalso (sodium cycle), Zeo-Dur (sodium cycle), or De-Acidite. For IR-4B, 0.439-gm. samples were used. The amount of regulators adsorbed was determined, using procedures described for static trials (table 2).

Data of table 2 indicate that IR-100 H and Zeo-Karb H adsorbed much of the growth-regulators, while the Decalso and Zeo-Dur adsorbed little or none. The anion exchangers, especially IR-4B, adsorbed much of the growth-regulators. The IR-100 H adsorbed almost equal micro-equivalents of 2,4-D and its salts. De-Acidite and IR-4B, however, adsorbed less of Cu(2,4-D)_2 than of 2,4-D, Ca(2,4-D)_2 , or $\text{NH}_4\text{2,4-D}$. IR-100 H adsorbed less of 2,4,5-T than of 2,4-D or its salts but adsorbed much more IPPC. With the anion exchangers the situation was reversed; there was a relatively large amount of 2,4,5-T adsorbed and a small amount of IPPC.

The results of this experiment showed that growth-regulators were strongly adsorbed by cation exchangers in the hydrogen cycle but that little or no adsorp-

TABLE 2
ADSORPTION OF SIX PLANT GROWTH-REGULATORS BY SIX EXCHANGER MATERIALS
EACH FIGURE IS AN AVERAGE OF THREE REPLICATES

Growth-regulator	Micro-equivalents adsorbed	Mg. adsorbed per ½ gm. oven-dry exchanger	% adsorbed	Micro-equivalents adsorbed	Mg. adsorbed per ½ gm. oven-dry exchanger	% adsorbed	Micro-equivalents adsorbed	Mg. adsorbed per ½ gm. oven-dry exchanger	% adsorbed
	IR-100 H			Zeo-Karb H			Decalso		
2,4-D.....	5.78	1.46	51.1	11.0	2.58	97.3	0	0	0
NH ₄ 2,4-D.....	5.97	1.62	52.8	11.0	2.78	97.3	0	0	0
Cu(2,4-D) ₂	5.72	1.64	50.6	11.0	2.93	97.3	0	0	0
Ca(2,4-D) ₂	6.13	1.68	54.2	11.1	2.82	98.1	0.27	0.70	2.39
2,4,5-T.....	4.77	1.39	42.2	11.0	2.98	97.3	0	0	0
IPPC.....	8.70	1.78	76.9	11.2	2.13	99.0	0.45	0.87	3.98
	Zeo-Dur			De-Acidite			IR-4B		
2,4-D.....	0	0	0	5.23	1.83	46.2	9.05	2.53	80.0
NH ₄ 2,4-D.....	0.16	0.041	1.41	5.08	1.91	44.9	8.45	2.55	74.7
Cu(2,4-D) ₂01	.003	0.09	3.42	1.36	30.2	7.70	2.46	68.1
Ca(2,4-D) ₂18	.047	1.59	5.49	2.08	48.5	8.60	2.62	76.0
2,4,5-T.....	.73	.201	6.45	6.17	2.49	54.6	9.21	2.99	81.4
IPPC.....	0.43	0.083	3.80	1.03	0.29	9.1	5.47	1.24	48.4

tion occurred with those in the sodium cycle. Another experiment was performed to determine how much 2,4-D or IPPC would be adsorbed by IR-100 in the hydrogen, sodium, or calcium form. It was shown that much IPPC is adsorbed by all exchangers but that only the hydrogen form adsorbed much 2,4-D (table 3). Each test was made in duplicate.

Another experiment was run to determine whether a hydrogen exchanger adsorbs 2,4-D mainly by virtue of its properties as an acid. Fifty-milliliter aliquots containing 40 p.p.m. of 2,4-D in the presence of varying amounts of hydrochloric acid were added to 0.5-gm. samples of IR-100 H. The solutions were adjusted to five different degrees of acidity. A series of blanks was run with acidulated water adjusted to the same pH as the solutions of 2,4-D. Much less 2,4-D was adsorbed

when the pH of the solution was 3.3 than when it was 2.5 or lower (table 4).

In another experiment in which varying concentrations of 2,4-D were added

TABLE 3
ADSORPTION OF 2,4-D AND IPPC BY IR-100 H, IR-100 Na, AND IR-100 Ca FROM 0.000226 N AQUEOUS SOLUTIONS OF THE COMPOUNDS

GROWTH-REGULATOR	% OF REGULATOR ADSORBED		
	IR-100 H	IR-100 Na	IR-100 Ca
2,4-D.....	57.9	0.44	3.2
IPPC.....	81.4	74.50	70.0

to 0.5-gm. samples of IR-100 H or IR-4B, the amount of 2,4-D adsorbed by the ion exchangers was approximately in direct proportion to the initial concentration of 2,4-D (table 5). The percentage of 2,4-D adsorbed was about the same at all concentrations used.

It was attempted to elute 2,4-D from IR-100 H with 0.75 *N* solutions of HCl, NaCl, CaCl₂, or AlCl₃ · 6H₂O. The 2,4-D was put on the exchanger with alcohol so that there was about 1.5 mg. of 2,4-D per 0.5 gm. of air-dry resin. The solutions were also added to exchangers which contained no 2,4-D. The latter were used as blanks. Each test was run in duplicate. It was found that the presence of the salts decreased the quantity of 2,4-D eluted as compared with that eluted by distilled water (table 6). Hydrochloric acid had little effect upon the amount of elution. Since it was thought that the method of application of 2,4-D to the ex-

TABLE 4
ADSORPTION OF 2,4-D BY IR-100 H IN
THE PRESENCE OF HYDRO-
CHLORIC ACID

Initial pH	% of 2,4-D adsorbed
3.3.....	52.4
2.5.....	89.4
2.0.....	89.4
1.7.....	93.2
1.4.....	89.4

changer with alcohol may have affected its exchange properties, some 2,4-D was applied to the exchanger in aqueous solution. When the exchanger containing 2,4-D was shaken with a 0.75 *N* solution of sodium chloride, results similar to those shown in table 6 were obtained.

Elution of 2,4-D from ion exchangers by use of anions of varying valences was attempted. Solutions of 0.685 normality of NaCl, Na₂SO₄, and Na₃PO₄ · 12H₂O were prepared and were added to IR-100 H or Zeo-Karb H containing, respectively, 1.5 or 2.5 mg. of 2,4-D per 0.5 gm. of air-dry exchanger. Each test was made in duplicate, and blanks were run on exchangers containing no 2,4-D. Less 2,4-D was eluted from IR-100 H by the salts than by distilled water, and, the higher the valence of the anion, the smaller was the amount of 2,4-D eluted (table

7). No 2,4-D was eluted from Zeo-Karb H.

It was shown that IPPC could be eluted from IR-100 H by 0.685 *N* sodium chloride. The growth-regulator was applied to IR-100 H or to Zeo-Karb H in alcohol so that each contained, respectively, 1.5 or 2.5 mg. of IPPC per 0.5 gm. of air-dry exchanger. The sodium chloride solution eluted 33.3% of the growth-regulator from IR-100 H, while water eluted

TABLE 5
ADSORPTION OF 2,4-D BY IR-100 H AND
IR-4B FROM SOLUTIONS OF VARY-
ING CONCENTRATION

Exchanger	Initial conc. of 2,4-D (p.p.m.)	Mg. 2,4-D adsorbed by ½-gm. oven-dry exchanger	% adsorbed
IR-100 H.....	20	0.63	63.0
	50	1.46	58.4
	100
	200	6.85	68.5
IR-4B.....	20	0.89	89.0
	50	2.06	82.4
	100	4.09	81.8
	200	7.97	79.7

none. No IPPC was eluted from Zeo-Karb H.

The following experiment shows that 2,4-D is readily eluted from the anion exchangers, IR-4B and De-Acidite, by a suitable concentration of hydrochloric acid. IR-4B and De-Acidite were prepared which contained, respectively, 1.39 and 1.16 mg. of 2,4-D per 0.5 gm. of air-dry exchanger. To 0.5-gm. samples of the resins were added 0.126 or 0.00126 *N* hydrochloric acid. The stronger concentration of acid readily replaced the adsorbed 2,4-D (table 8).

LEACHING EXPERIMENTS

Static trials showed that 2,4-D was strongly adsorbed by IR-100 H and Zeo-

Karb H and that the compound was not readily eluted from the exchangers by water or salt solutions. It was further shown that Decalso and Zeo-Dur adsorbed little or no 2,4-D. Since many soils may have a low adsorptive capacity for 2,4-D, an experiment was set up to determine how readily 2,4-D is leached from Decalso and Zeo-Dur.

The sodium forms of the exchangers were used. The 2,4-D was applied to the exchangers at two rates: 19.4 and about 64 mg. per pound on a dry-weight basis. Beds of Decalso 10 cm. deep contained 3 or 10 mg. of 2,4-D while beds of Zeo-Dur contained 5.17 or 17.0 mg. Some tubes used as blanks contained beds with no 2,4-D. Each bed received three successive leachings of 55 ml. of distilled water. The second and third leachings of the beds of Decalso were made 2 and 4 hours after the first; with Zeo-Dur, in about 24 and 48 hours after the first.

Much 2,4-D was removed by the first leaching, a smaller amount by the second leaching, while practically none was found in the third leachate (table 9). In

TABLE 6

ELUTION OF 2,4-D FROM 0.5-GM. SAMPLES OF IR-100 H BY WATER AND BY 0.75 *N* SOLUTIONS CONTAINING A MONO-, DI-, OR TRIVALENT CATION

Solution	Mg. of 2,4-D eluted	% of 2,4-D eluted
Water.....	0.685	45.7
HCl.....	.720	48.0
NaCl.....	.335	22.3
CaCl ₂370	24.7
AlCl ₃ ·6H ₂ O.....	0.436	29.1

no case was more than 90% of the 2,4-D eluted by the 6 inches of water applied, and usually the percentage was much less.

An experiment was run to compare the rate of leaching of six growth-regulators

from Decalso and Zeo-Dur. IPPC, 2,4-D, the ammonium, calcium, and cupric salts of 2,4-D, and 2,4,5-T were the compounds used. All compounds except IPPC were applied to Decalso at the rate of 600 micro-equivalents per pound and

TABLE 7

ELUTION OF 2,4-D FROM 0.5-GM. SAMPLES OF IR-100 H BY WATER AND BY 0.685 *N* SOLUTIONS CONTAINING A MONO-, DI-, OR TRIVALENT ANION

Solution	Mg. of 2,4-D eluted	% of 2,4-D eluted
Water.....	0.976	65.1
NaCl.....	.420	28.0
Na ₂ SO ₄	0.385	25.7
Na ₃ PO ₄ ·12H ₂ O.....	0	0

to Zeo-Dur at the rate of 342 micro-equivalents per pound. IPPC was applied to Decalso and Zeo-Dur at rates of 660 and 376 micro-equivalents per pound, respectively. A bed of each type of exchanger contained 102.3 micro-equivalents of IPPC or 93.0 micro-equivalents of one of the other compounds.

Each bed was leached with 2 inches of water. Usually more than one-half of each regulator was leached from each exchanger (table 10). All compounds were leached from the exchanger materials in about the same amounts. An exception was IPPC on Zeo-Dur, which was leached to a much lower degree.

PLANT GROWTH IN ION EXCHANGERS

Experiments were conducted to determine the toxicity to plants of NH₄2,4-D adsorbed on exchange materials mixed in soil in which they were grown. In one experiment the emergence of seedlings from soils containing NH₄2,4-D adsorbed on a strong adsorbent (Zeo-Karb H) or on a weak adsorbent (quartz sand) was studied. One hundred

milliliters of 2.0, 0.2, 0.02, or 0.002% aqueous solutions of $\text{NH}_4\text{2,4-D}$ were added to 60 gm. of air-dry Zeo-Karb H or to quartz sand in 250-ml. beakers. The mixtures were stirred occasionally over a 2-hour period and then were dried in an

tard were planted in each pot. Each treatment consisted of three replicate pots.

Seven days after planting, counts were made of seedlings appearing above the soil surface (table 11). The data indicate that there was only a limited or no toxicity to the germinating seedlings from the low concentrations of $\text{NH}_4\text{2,4-D}$ adsorbed on Zeo-Karb H but that toxicity was strong at the high concentration. Perhaps much of the $\text{NH}_4\text{2,4-D}$ was strongly adsorbed by the ion exchanger, and the growth-regulator in an adsorbed condition could not affect plant growth. White mustard germinated normally in soil-Zeo-Karb H mixtures containing $\text{NH}_4\text{2,4-D}$ at a rate of 10 mg. per pound of soil, but, when sand only was used as the carrier for the growth-regulator, the lowest concentration (1 mg. / lb.) prevented germination. Barley germinated well in soil-Zeo-Karb H mixtures when $\text{NH}_4\text{2,4-D}$ was present at the concentration of 100 mg. per pound, but with sand as the carrier 10 mg. of $\text{NH}_4\text{2,4-D}$ per pound of soil reduced the number of emerging barley seedlings.

In another experiment the growth of plants in exchangers containing high con-

TABLE 8

ELUTION OF 2,4-D FROM IR-4B AND DE-ACIDITE BY HYDROCHLORIC ACID

SOLUTION	IR-4B		DE-ACIDITE	
	Mg. of 2,4-D eluted	% of 2,4-D eluted	Mg. of 2,4-D eluted	% of 2,4-D eluted
Water.....	0.000	0	0.03	0.02
0.00126 <i>N</i> HCl....	0	0	0.03	0.02
0.126 <i>N</i> HCl.....	0.578	41.6	1.10	94.83

oven at 50° C. Each sample was then thoroughly mixed with about 1.87 lb. of a potting soil (three parts soil to one part fine gravel). Thus mixtures of soil and Zeo-Karb H or sand were obtained which contained about 0, 1, 10, 100, or 1,000 mg. of $\text{NH}_4\text{2,4-D}$ per pound of soil when calculated on an air-dry basis. The soils were placed in 4-inch unglazed clay pots, which were set on a greenhouse bench. Fifteen seeds of barley and white mus-

TABLE 9

LEACHING OF 2,4-D FROM DECALSO AND ZEO-DUR BY 2-INCH COLUMNS OF WATER. FIGURES ARE AVERAGES OF THREE REPLICATES

EXCHANGER	MG. OF 2,4-D/LB.	LEACHING					
		First		Second		Third	
		Mg. of 2,4-D eluted	% of 2,4-D eluted	Mg. of 2,4-D eluted	% of 2,4-D eluted	Mg. of 2,4-D eluted	% of 2,4-D eluted
Decalso.....	19.4 64.7	0.960 4.070	32.00 40.70	0.528 0.950	17.60 9.50	0.011 .055	0.37 .55
Zeo-Dur.....	19.4 63.8	2.900 7.900	56.09 46.47	1.600 3.000	30.95 17.65	.025 0.055	.48 0.32

centrations of adsorbed $\text{NH}_4\text{2,4-D}$ was observed. Aqueous solutions of $\text{NH}_4\text{2,4-D}$ were added to Zeo-Karb H and IR-100 H, and the mixtures were dried at 70°C . The dried materials were then thoroughly mixed with two parts by

mustard plants had developed two true leaves in control gravel-Zeo-Karb H mixtures. Mustard plants also developed in mixtures containing 1 or 10 mg. of $\text{NH}_4\text{2,4-D}$ per pound. The barley in control mixtures was in the second leaf stage

TABLE 10

LEACHING OF SIX GROWTH-REGULATORS FROM DECALSO AND ZEO-DUR BY A 2-INCH COLUMN OF WATER. FIGURES ARE AVERAGES OF THREE REPLICATES

COMPOUND	INITIAL CONC. PER BED (MICRO-EQ.)	DECALSO			ZEO-DUR		
		Micro-eq. eluted	Mg. eluted	% eluted	Micro-eq. eluted	Mg. eluted	% eluted
2,4-D.....	93	54.8	12.11	58.9	62.3	13.77	67.0
$\text{NH}_4\text{2,4-D}$	93	52.5	12.50	56.5	60.6	14.42	65.2
Ca(2,4-D)_2	93	49.8	11.95	53.5	47.8	11.47	51.4
Cu(2,4-D)_2	93	56.2	14.15	60.4	54.2	13.64	58.3
2,4,5-T.....	93	51.7	13.21	55.6	45.2	11.55	48.6
IPPC.....	102.3	60.0	10.74	58.7	29.2	5.23	28.5

TABLE 11

AVERAGE NUMBER OF WHITE MUSTARD AND BARLEY SEEDLINGS PER POT EMERGED FROM SOILS CONTAINING VARIOUS AMOUNTS OF $\text{NH}_4\text{2,4-D}$ APPLIED TO SOIL ON ZEO-KARB H OR QUARTZ SAND. THREE REPLICATES

PLANT	CARRIER	CONCENTRATION OF $\text{NH}_4\text{2,4-D}$ (MG./LB.)				
		0	1	10	100	1,000
White mustard.....	{Zeo-Karb H	9.0	9.7	10.3	1.0	0.0
	{Sand	10.7	0.0	0.0	0.0	0.0
Barley.....	{Zeo-Karb H	11.0	6.7	8.3	9.7	0.0
	{Sand	9.0	7.3	3.3	0.3	0.0

weight of fine gravel. The gravel-IR-100 H mixture contained 0, 3, 30, or 300 mg. of $\text{NH}_4\text{2,4-D}$ per pound, and the gravel-Zeo-Karb H mixture 0, 1, 10, 100, or 1,000 mg. of $\text{NH}_4\text{2,4-D}$ per pound on a dry-weight basis. The mixtures were placed in 4-inch unglazed clay pots, and fifteen seeds of barley and white mustard were planted in each pot.

The plants were harvested 19 days after planting (fig. 2, table 12). White

and had an average foliage height of 6 inches. It had developed well in all gravel-Zeo-Karb H mixtures except those containing 1,000 mg. of growth-regulator per pound of mixture. The stubby roots of the barley growing in the latter mixture were usually less than 1 cm. in length (fig. 3).

White mustard failed to germinate in the gravel-IR-100 H mixture. The average fresh weights of tops of barley on a

one-pot basis for control gravel-IR-100 H mixture and for mixtures containing 3, 30, or 300 mg. of $\text{NH}_4\text{2,4-D}$ per pound were 1.59, 1.55, 0.27, and 0.09 gm., respectively. The root systems of barley in the mixtures treated with $\text{NH}_4\text{2,4-D}$ at a rate of 30 mg. per pound were small, and many of the roots were ribbon-like (fig. 3). The relative growth of barley in the gravel-Zeo-Karb H and gravel-IR-100 H mixtures indicates that $\text{NH}_4\text{2,4-D}$ is less toxic when adsorbed on Zeo-Karb H than on IR-100 H. This is probably asso-

ciated with the greater adsorptive capacity of Zeo-Karb H for $\text{NH}_4\text{2,4-D}$ than is possessed by IR-100 H.

Discussion

Cation exchangers in the hydrogen cycle strongly adsorbed all the growth-regulators studied. For example, IR-100 H adsorbed 2,4-D from a 0.02% solution at a rate of more than 6 gm. per pound of exchanger. It seems probable that when 2,4-D or its salts are added to soils in chemically equivalent quantities, ap-



FIG. 2.—White mustard and barley plants grown in mixtures of (*upper*) Zeo-Karb H and gravel and (*lower*) Amberlite IR-100 H and gravel. *Left to right*: Zeo-Karb H mixtures contained 0, 1, 10, 100, and 1,000 mg. of $\text{NH}_4\text{2,4-D}$ per pound; Amberlite mixtures contained 0, 3, 30, and 300 mg. of $\text{NH}_4\text{2,4-D}$ per pound. White mustard seedlings grew normally in Zeo-Karb H mixtures containing 10 mg. of $\text{NH}_4\text{2,4-D}$ per pound, and barley made much growth in Zeo-Karb H mixtures containing 100 mg. of growth-regulator per pound. Photographed 19 days after planting.

proximately equal amounts of the anion of each would be adsorbed by the soil. Such equivalent adsorption might not hold true in soils containing anion- or acid-exchanging materials in large amounts, as less $\text{Cu}(\text{2,4-D})_2$ was adsorbed by the anion exchangers than was 2,4-D or its ammonium or calcium salts.

The high adsorptive capacities of certain ion exchangers for growth-regulators, combined with the fact that salt solutions failed to elute all the 2,4-D from cation exchangers, may be of great significance when growth-regulators are applied as herbicides. When the adsorp-

TABLE 12
AVERAGE FRESH WEIGHTS (GM.) PER POT OF TOPS
OF BARLEY AND WHITE MUSTARD GROWN IN
GRAVEL-ZEO-KARB H MIXTURES CONTAIN-
ING VARIOUS CONCENTRATIONS OF $\text{NH}_4\text{2,4-D}$.
BASED ON THREE REPLICATES

PLANT	CONC. OF $\text{NH}_4\text{2,4-D}$ (MG./LB.)				
	0	1	10	100	1,000
White mus- tard.....	0.63	0.68	0.23	0.02	0.00
Barley.....	1.96	2.05	2.28	1.36	0.22

tive capacity of a soil is high, there may be less loss of the compound from the

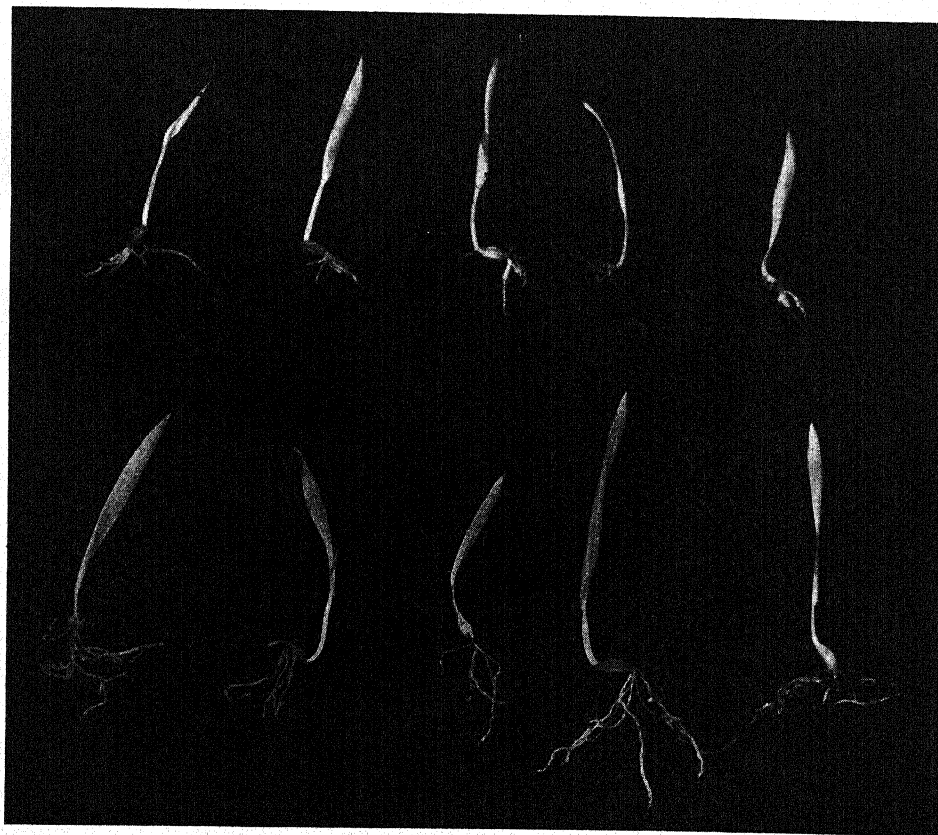


FIG. 3.—Barley seedlings grown in mixtures of (*upper*) Zeo-Karb H and gravel containing 1,000 mg. of $\text{NH}_4\text{2,4-D}$ per pound and of (*lower*) Amberlite IR-100 H and gravel containing 30 mg. of growth-regulator per pound. Roots in Zeo-Karb H mixtures were short and stubby. Many roots in Amberlite mixtures were flattened and had many small gall-like protuberances.

soil by leaching. But, when adsorbed, such material may be not particularly toxic to plants grown in the soil. For example, it was shown that much $\text{NH}_4\text{2,4-D}$ adsorbed on Zeo-Karb H was nontoxic to plants growing in mixtures of the ion exchanger and gravel. Barley plants grew well in gravel-Zeo-Karb H mixtures containing 100 mg. of $\text{NH}_4\text{2,4-D}$ per pound of mixture. The growth-regulator was evidently strongly adsorbed, since a surface application of $\text{NH}_4\text{2,4-D}$ of 100 mg. per 12.5 sq. in. (approximate surface area of a 4-inch clay pot) corresponds to a rate of about 110 pounds per acre, which is far in excess of any field application. It is possible that the compound did not come into contact with the roots growing around the adsorbent or perhaps could not diffuse into or enter the plants.

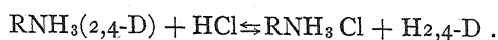
The varying toxicity of a growth-regulator in different soils may be associated with the differences in adsorptive capacities of the soils for the various regulators. Since adsorptive capacities of soils may affect toxicity of a growth-regulator in them, it is evident that any modification of such capacities through agricultural practices might be very important. Because soils are very complex systems, it is difficult to say which factors are most concerned with the degree of toxicity and with the fate of a growth-regulator in a soil. In a soil of high adsorptive capacity, one would expect greater retention (less leaching) of the compound, but the amount present in such a condition as to affect plant growth might not necessarily be correspondingly high. Much of the adsorbed compound might be nontoxic to plants. The rate of decomposition of the compounds in soil is of great importance. It would be of interest to know whether adsorbed compounds are subject to the same decom-

position processes and rates as the non-adsorbed. A desirable carrier for 2,4-D for certain uses might be one with a high adsorptive capacity which would slowly release the compound over a long period of time.

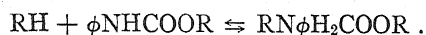
The effectiveness of any herbicidal practice involves many details regarding herbicides, crops, and soils. The fate of a growth-regulator in soil is probably just as much influenced by agricultural practices as is the amount and availability of nutrients and the activity of organic constituents. Thus many interlocking factors may determine the toxicity and duration of a growth-regulator in a soil. By the use of a suitable system of soil management, the toxicity and persistence of a compound in the soil may be modified. The indication is that acidic soils might have higher adsorptive capacities for 2,4-D or its salts than alkaline ones. Cation exchangers in the sodium or calcium form adsorbed little or no 2,4-D. The respiration of plant roots and the microflora in a soil may result in changes in the soil pH during the season which might affect adsorption of growth-regulators. Soil reaction can be changed by application of ammonium sulfate, lime, or other compounds. It is possible that various compounds may be found in the soil which would elute growth-regulators from the soil adsorbents and would also change the degree of solubility of the herbicides in the soil solution. The persistence of toxicity of growth-regulators in soil may be partially controlled by addition of organic matter or by liming. KRIES (4) has shown that addition of lime to soil decreased the rate of disappearance of the toxic effects of 2,4-D, while the addition of organic matter counteracted the effect of the lime.

A chemical or physical explanation of the adsorption of 2,4-D and its salts by

the cation exchangers is difficult with the data available. 2,4-D is probably eluted from anion exchangers in a typical acid or anion exchange reaction, since 2,4-D is readily eluted by hydrochloric acid:



IPPC differs from 2,4-D in that much more IPPC is adsorbed by cation exchangers, and much less by anion exchangers, and it may be eluted from IR-100 H by sodium chloride. It is also adsorbed by the sodium and calcium forms of IR-100. IPPC may react with IR-100 H by physical adsorption or by salt formation with the basic amino group:



The experiments in leaching using beds of Decalso and Zeo-Dur showed that the growth-regulators are readily leached from exchange materials having little or no adsorptive capacity. The various compounds were usually removed in about the same amounts. Even the most soluble compound used, $\text{NH}_4,2,4\text{-D}$, was not removed in greater quantities than 2,4-D, which is of much lower solubility. Leaching experiments in which smaller quantities of water were used might result in differential rates of leaching of the various compounds based on their solubilities. It seems probable, however, that in the soil 2,4-D or a relatively insoluble 2,4-D salt might be converted to soluble forms by reaction with ammonium, sodium, or other ions present in the soil solution.

Experiments in which exchanger beds were successively leached with 2-inch columns of water showed that 4 inches of water removed practically all the 2,4-D that could be leached from the beds. In the case of Decalso beds this amounted to only about one-half of the total amount

of the growth-regulator initially in the bed. It may be that the Decalso adsorbed some 2,4-D because of the method of application of the compound to the exchanger. The important point, however,

is the rapidity with which 2,4-D was removed from the beds by leaching.

Summary

1. Studies were made on the adsorption by and elution from several ion exchangers of six plant growth-regulators. Cation exchangers in the hydrogen cycle adsorbed much 2,4-dichlorophenoxyacetic acid (2,4-D) and its ammonium, calcium, and cupric salts, and in about equal amounts. Anion exchangers adsorbed much 2,4-D and its salts, but not always in equal amounts. Little or no 2,4-D or its salts was adsorbed by cation exchangers in the sodium or calcium form. 2,4,5-Trichlorophenoxyacetic acid was adsorbed in amounts similar to those of 2,4-D except that less of the former was adsorbed by the cation exchangers.

2. Isopropyl N-phenylcarbamate was more strongly adsorbed than 2,4-D by cation exchangers in the hydrogen, sodium, or calcium form. Much less was adsorbed by the acid exchangers.

3. Much less 2,4-D was adsorbed by Amberlite IR-100 H when the pH of the solution was 3.3 than when it was 2.5 or lower. The amount of 2,4-D adsorbed by ion exchangers from solutions varying in concentration from 20 to 200 p.p.m. was in about direct proportion to the concentration of 2,4-D in the initial solution.

4. Solutions of NaCl, CaCl_2 , or $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ of 0.75 normality caused less elution of 2,4-D adsorbed on Amberlite IR-100 H than did water. The amounts eluted by hydrochloric acid and water

were approximately equal. Solutions of NaCl, Na₂SO₄, and Na₃PO₄·12 H₂O of 0.685 normality also eluted less 2,4-D from Amberlite IR-100 H than did water. Isopropyl N-phenylcarbamate, however, was eluted from IR-100 H by sodium chloride solution.

5. Hydrochloric acid eluted 2,4-D from anion exchangers, probably in typical anion- or acid-exchange reactions.

6. 2,4-D was mixed with Decalso and Zeo-Dur, respectively, at rates of 19.4 or about 64 mg. per pound. The mixtures were leached with three 2-inch columns of water. Much 2,4-D was removed by the first leaching, a smaller amount at the second leaching, and practically none at the third leaching.

7. About 93 micro-equivalents of six different growth-regulators were mixed with Decalso and Zeo-Dur and the mix-

tures were leached with a 2-inch column of water. One-half or more of each compound was usually leached from the exchanger materials, and the different compounds were usually removed in about equivalent amounts.

8. Growth of plants in soils containing ion exchangers upon which NH₄2,4-D was adsorbed showed that much of adsorbed growth-regulator is nontoxic. Barley and white mustard plants grew well in gravel-Zeo-Karb H mixtures containing NH₄2,4-D at concentrations of 100 and 10 mg., respectively, per pound of mixture. The growth-regulator may not have come into contact with the roots growing around the adsorbent or perhaps could not diffuse into or enter the plants.

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EMBRYOLOGY OF CEPHALOSTIGMA SCHIMPERI

S. B. KAUSIK AND K. SUBRAMANYAM

Introduction

The genus *Cephalostigma* A. DC. belongs to the family Campanulaceae, tribe Campanuloideae, subtribe Wahlenbergineae (4). GAMBLE (5) records three species in South India: *C. schimperi* Hochst., *C. flexuosum* Hook., and *C. hookeri* C. B. Clarke. The present investigation deals with *C. schimperi* only.

The literature on the embryology of the family has already been reviewed by SCHNARF (14) and by KAUSIK and SUBRAMANYAM (9) and need not be covered again.

Material and methods

The material was collected from three sources: Government Botanical Gardens, Lalbagh, Bangalore; Bannerghatta (near Bangalore); and Nandi Hills (a hill station 35 miles from Bangalore). It was fixed in Allen's modification of Bouin's fluid and in formalin-acetic-alcohol, both of which were quite satisfactory. Sections cut at a thickness of 10-20 μ were stained in Heidenhain's iron-alum haematoxylin with eosin as a counterstain.

Investigation

MICROSPOROGENESIS AND MALE GAMETOPHYTE

The wall of the anther (fig. 1) is made up of three layers external to the tapetum. The outermost is the epidermis; next is the endothecium, which acquires the usual fibrous thickenings in later stages; and then an ephemeral layer which is soon disorganized. The tapetal cells are at first uninucleate but later become binucleate and also begin to show conspicuous vacuoles.

The microspore mother cells undergo the usual reduction divisions and form tetrads of microspores (figs. 2-4) which are usually tetrahedral but sometimes show an isobilateral arrangement. The separation of the microspores takes place by cleavage furrows.

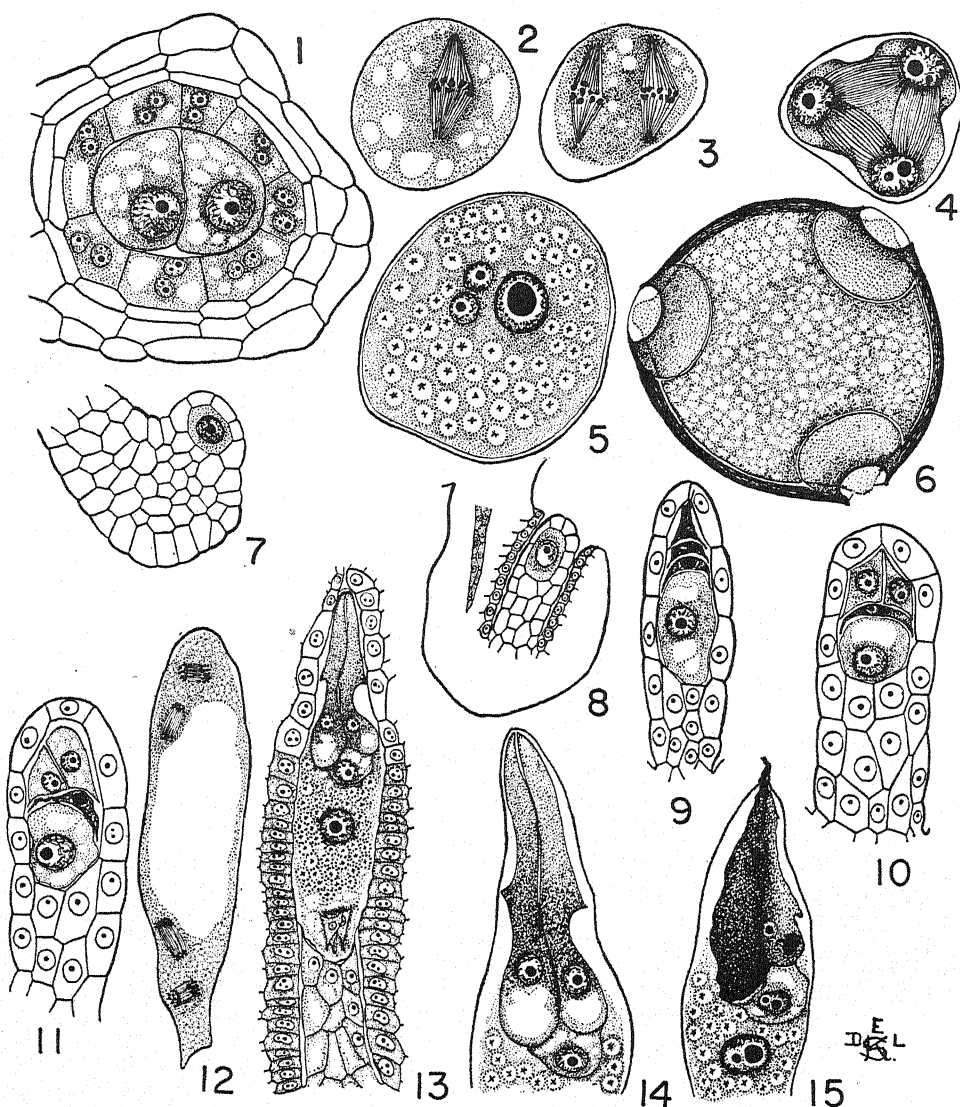
The mature pollen grain is packed with starch grains and contains three nuclei—a large tube nucleus and two small male nuclei (fig. 5). The exine is thick and rigid and shows reticulate thickenings on the surface (fig. 6). The intine appears as a thin delicate membrane. There are three conspicuous germ pores.

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

The ovary is inferior, tricarpeal, and trilocular, with numerous anatropous unitegmic ovules attached to axile placentae. The single hypodermal archesporial cell (fig. 7) functions directly as the megaspore mother cell (fig. 8). The megaspores may show a linear (fig. 9) or T-shaped (fig. 10) arrangement, and sometimes the micropylar dyad cell divides by an oblique wall (fig. 11). The three micropylar megaspores degenerate, and the chalazal one functions to give rise to a normal eight-nucleate embryo sac (figs. 12, 13).

The integument is well developed even at the megaspore mother cell stage (fig. 8). When the embryo sac is two- or four-nucleate, the nucellar epidermis becomes disorganized and the embryo sac comes in direct contact with the integumentary tapetum, which reaches its maximum development at the time of fertilization.

Meanwhile certain cells in the chalazal region of the nucellus become elongated



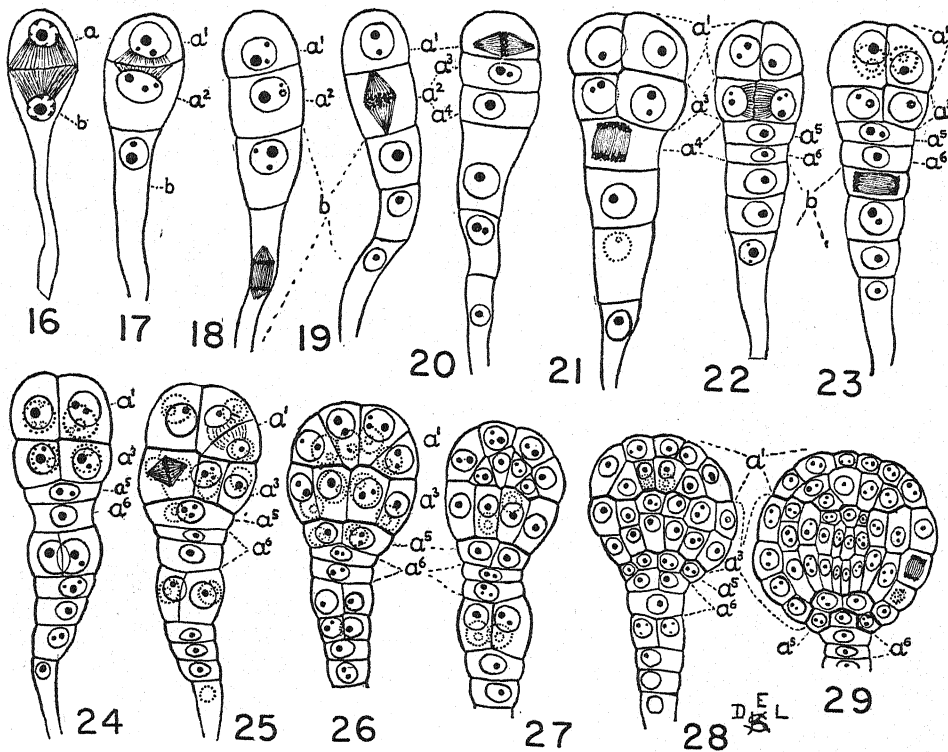
FIGS. 1-15.—Fig. 1, portion of cross section of young anther showing wall layers, binucleate tapetum, and microspore mother cells. $\times 900$. Figs. 2, 3, 4, tetrad divisions in microspore mother cell. $\times 1800$. Fig. 5, mature pollen grain showing trinucleate condition. Note starch grains in cytoplasm. $\times 1800$. Fig. 6, pollen grain in surface view showing three germ-pores and reticulate exine. $\times 1800$. Fig. 7, young ovule showing hypodermal archesporial cell and primordium of integument. $\times 600$. Fig. 8, anatropous ovule showing megaspore mother cell. $\times 280$. Fig. 9, linear tetrad of megaspores, the chalazal cell enlarging. $\times 900$. Fig. 10, T-shaped tetrad of megaspores. $\times 900$. Fig. 11, megaspore tetrad with oblique wall in upper dyad cell. $\times 900$. Fig. 12, third nuclear division from megaspore leading to formation of eight-nucleate stage. $\times 900$. Fig. 13, mature embryo sac showing egg apparatus, antipodal cells, and fusion nucleus; note starch grains. $\times 630$. Fig. 14, upper part of embryo sac showing elongated synergids and egg cell. $\times 900$. Fig. 15, stage in double fertilization showing remnants of pollen tube. $\times 900$.

and thickened and seem to serve a conducting function (figs. 8-11, 13).

The fully mature embryo sac is tapering at both ends (fig. 13). The synergids are elongated structures with pointed beaklike apices. The pear-shaped egg cell

(11), this degeneration is postponed to a slightly later stage and takes place during the early stages of endosperm development.

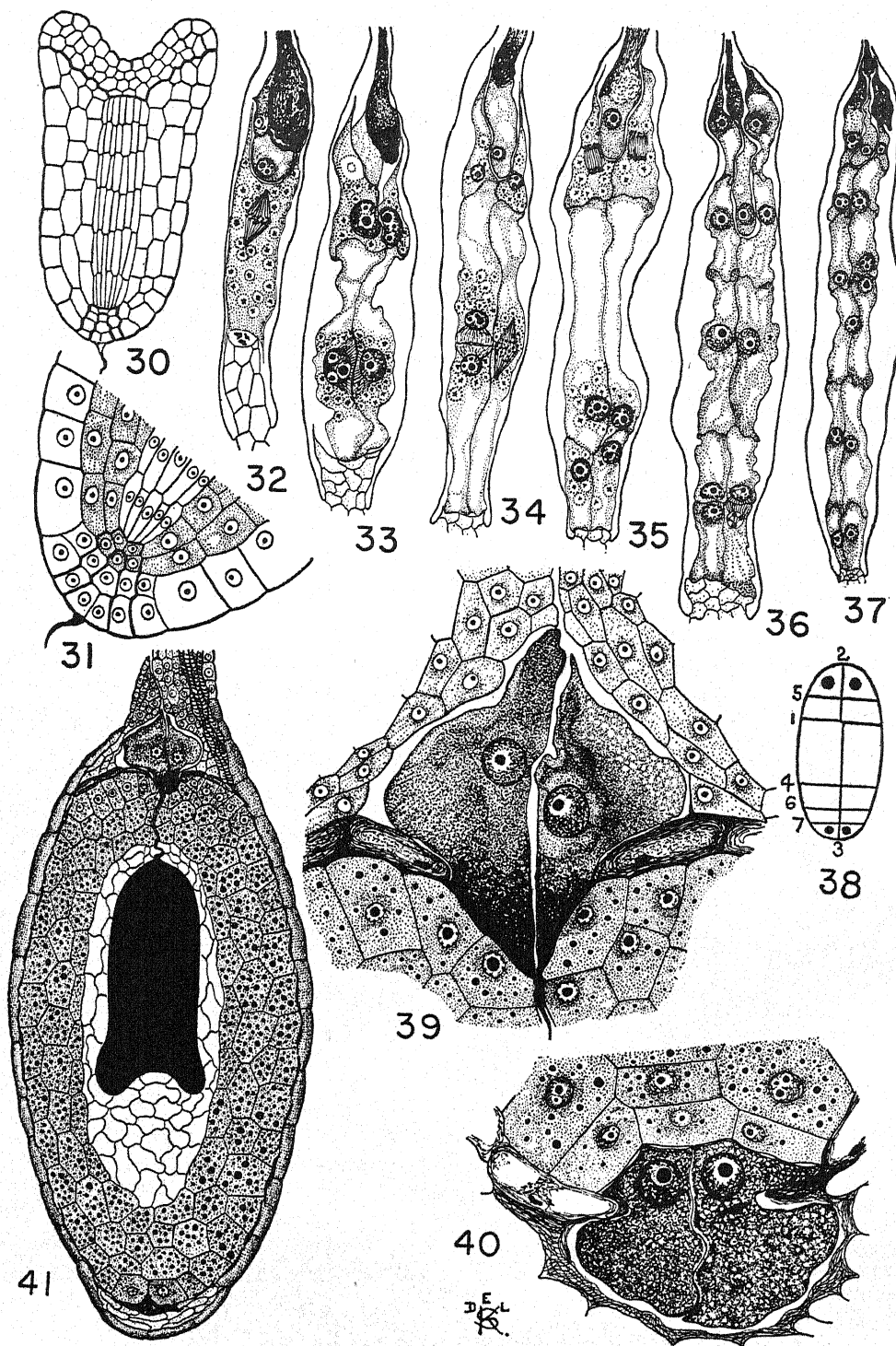
A characteristic feature of the embryo sac is the presence of a large number of



FIGS. 16-29.—Stages in development of embryo. Primary segmentation walls are indicated by thicker lines. All $\times 900$, except figs. 28, 29, $\times 630$.

is situated between them and has a conspicuous nucleus. The two polar nuclei meet near the center of the embryo sac and fuse to form the fusion nucleus. The antipodal cells taper at their lower ends and thus offer a marked resemblance to those of the allied family Lobeliaceae (8, 9, 10). They degenerate at about the time of fertilization. In another member of the same family, *Sphenoclea zeylanica* (12), and in one of the Lobeliaceae, *Lobelia nicotianaefolia*

starch grains. Usually they appear after the fusion of the two polar nuclei (figs. 13-15) and persist during the early stages of endosperm development, being crowded around the nucleus of each endosperm cell (figs. 32-35). DAHLGREN (2, 3), who has reviewed the occurrence of starch grains in embryo sacs, lists *Campanula rotundifolia* as an instance where the starch appears at the two-nucleate stage but becomes considerably reduced in quantity in older stages.



FIGS. 30-41.—Fig. 30, embryo showing cotyledons; note collapsed suspensor. $\times 270$. Fig. 31, basal portion of mature embryo enlarged to show root-cap, dermatogen, periblem (stippled), and plerome. $\times 270$.
(Legend continued on following page)

The pollen tube enters the embryo sac by destroying one of the synergids. Double fertilization takes place normally (fig. 15).

EMBRYO

The development of the embryo closely corresponds to that described for *Campanula patula* (15), *Lobelia syphilitica* (1), and *L. amoena* (6). Stages in development are presented in figures 16-31.

ENDOSPERM

The primary endosperm nucleus, which lies near the zygote, divides (fig. 32), and the accompanying development of a transverse wall gives rise to a small primary micropylar and a larger primary chalazal chamber. Next a vertical wall is formed in each of these chambers, resulting in a four-celled stage (fig. 33). A transverse division now follows in the chalazal pair of cells (fig. 34) and subsequently in the micropylar pair (fig. 35), resulting in the formation of four tiers of paired cells. The two cells of the first tier now form the micropylar haustorium, and those of the lowest tier give rise to the chalazal haustorium after undergoing one transverse division (fig. 36) or perhaps two (fig. 37). The remaining tiers of cells, which lie between the haustoria, undergo further transverse and longitudinal divisions and give rise to the endosperm. Thus the sequence of wall formation (fig. 38) closely corresponds

with that in the "Codonopsis type" found in other members of the Campanulaceae (13).

The micropylar haustorium (fig. 39) is two-celled, each cell forming a prominent lateral hump and containing a conspicuous nucleus imbedded in a dense mass of finely vacuolate cytoplasm. Being situated close to the rich nutritive tissue of the micropylar part of the integument, this haustorium remains active for a long time. The chalazal haustorium (fig. 40) consists of two bulbous cells which digest their way into the chalazal tissues and are later seen as darkly stained, compressed structures lying in a mass of collapsed cells.

All the cells of the endosperm, except those lying in the immediate vicinity of the embryo, contain considerable quantities of starch. The seed coat is formed by the outermost layer of the integument. In mature seeds (fig. 41) the embryo, with its shriveled suspensor, is seen buried in a large mass of endosperm.

We wish to thank Dr. L. N. RAO, Central College, Bangalore, and Dr. P. MAHESHWARI, Dacca University, Dacca, for their help and encouragement. We are also grateful to Mr. S. N. CHANDRASEKHARA IYER, Coimbatore, for determining the specific name of the plant.

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Fig. 32, first division of primary endosperm nucleus. $\times 630$. Figs. 33-37, stages in development of endosperm and differentiation of micropylar and chalazal haustoria. Note aggregation of starch grains around nuclei of endosperm cells in figs. 32-35. Figs. 33-35, $\times 630$; fig. 36, $\times 450$; fig. 37, $\times 270$. Fig. 38, diagram showing sequence of wall formation in endosperm. Fig. 39, two-celled micropylar haustorium in advanced stage. $\times 900$. Fig. 40, two-celled chalazal haustorium in advanced stage. $\times 900$. Fig. 41, longitudinal section of mature seed, showing embryo with collapsed suspensor, starchy endosperm, persisting micropylar haustorium, remnants of chalazal haustorium, and thick-walled seed coat. $\times 200$.

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EFFECT OF A TEMPERATURE GRADIENT ON DISTRIBUTION OF WATER IN APPLES¹

MILTON A. LESSLER

Introduction

Earlier work on apples and other fruits subjected to a temperature gradient has shown that there is water movement from the warmer to the cooler side (3, 4). Visible evidence of this movement is a progressively increased wrinkling and then a necrosis of the warmer side, while there is an increase in the turgidity of the cooler side. In some cases, the cool side may become turgid enough to split the skin.

LORENZE and KNOTT (5) showed that gray-wall or thin-wall of tomato occurred only on the side of the fruit that was exposed to the sun. They found that the condition resulted from a lowered water

content on the exposed side, leading to the development of very small parenchyma cells on the inside of the pericarp. Temperature determinations of exposed and unexposed sides of the fruits *in situ* showed differences of as much as 15°-20° F. between the two sides.

REED and BARTHOLOMEW (6) made observations on young fruits of lemons, walnuts, apples, and tomatoes and found no intercellular spaces, but their observations led them to conclude that water passes over the surfaces of the cells as well as through them by osmosis. They concluded that in young fruits movement of water as a vapor is improbable but that water may move by capillarity through the hydrophylic colloids present in the cell walls.

ARTHUR (1) attributed the shriveling

¹ Work conducted at Cornell University, Department of Botany, under the direction of Dr. O. F. CURTIS.

and necrosis, in apples, to a specific infra-red injury but later (2) stated that it is possible that an equal amount of energy in the visible region would produce a similar injury. CURTIS (3), using methods similar to those of ARTHUR (1), found that the wrinkling and later necrosis of the warm side of the apple resulted from a movement of water from the warmer to the cooler side. Further stud-

All fruits were weighed before and after the gradient treatment and the total water loss noted. This loss varied but averaged only 28% of the actual difference between the cool and warm sides of the fruits and was of the same order as the total water loss from the controls.

After treatment, each fruit was cut into warm and cool halves on a pre-marked line and each half put on a

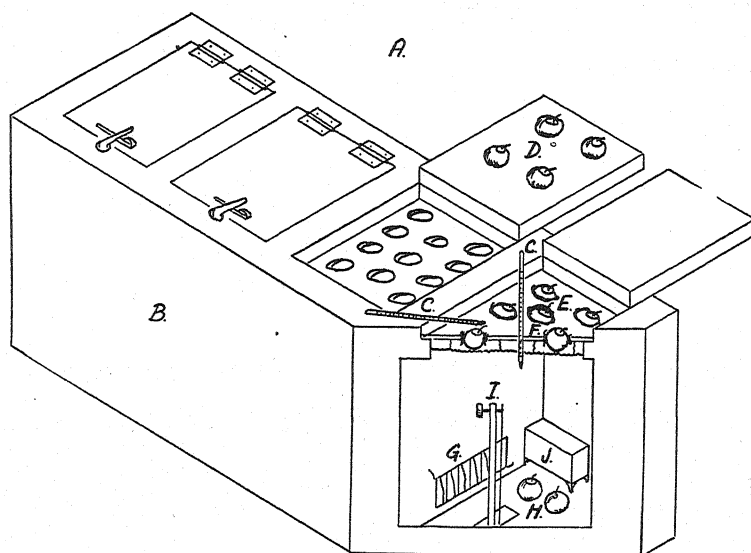


FIG. 1.—Apparatus used for gradient treatment. *A*, 6° C. refrigerator room; *B*, incubator; *C*, thermometers; *D*, cold control apples; *E*, asbestos panel with experimental apples; *F*, cotton packing around apple; *G*, heating unit; *H*, warm control apples; *I*, thermostat; *J*, thermograph.

ies on this problem will be presented in this report.

Experimentation

The apparatus for producing a temperature gradient was an incubator placed on its back, so that the doors were on the upper side (fig. 1), in a constant-temperature room kept at 6° C. The incubator doors were laid back, and carefully fitted asbestos panels were put into the openings. The fruits were fitted into holes in the panels, and cotton was carefully packed around them.

marked tared watch glass. After drying for 7 days in a blower oven at 65° C. and for 7 days in a vacuum oven at 60° C. the samples were again weighed. This drying treatment brought the halves to constant weight.

Figure 2 graphically shows the results from twenty-eight apples (variety Wealthy) given gradient treatment and eleven apples used as controls. The air on the warm side was kept at 25° C. and on the cool side at 6° C. Results from representative apples taken at intervals indicated a rapid movement of water (from

the warmer to the cooler side) for the first 3 days and then a tendency for the rate of movement to drop off.

In a determination of the relative distribution of water in apples after a $3\frac{1}{2}$ -day gradient treatment with the air on the warm side at 20°C . and on the cool side at 6°C ., plugs 1 cm. in cross section were cut both parallel and perpendicular to the stem-flower axis; each plug was cut transversely into three parts of equal length. To compensate for differences in water content that might occur

water was found in the middle portion of both experimental and control plugs, it is reasonable to assume that it was higher there at the start of the experiment, possibly because the two end portions of the plugs had skin on them, while there was none on the middle section.

Young Wealthy and Oldenburgh apples ($1\frac{1}{2}$ inches in diameter) were given a $3\frac{1}{2}$ -day gradient treatment (cool side 6°C ., warm side 25°C .). Visual observations, after the treatment, showed some

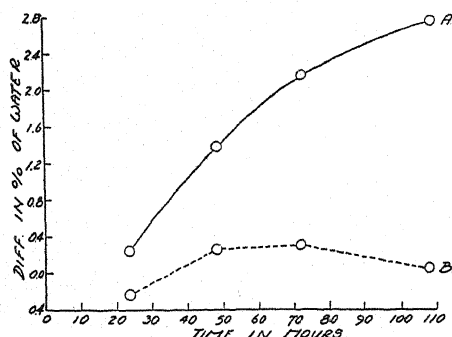


FIG. 2.—Movement of water in apples during $4\frac{1}{2}$ -day gradient treatment. A, difference in % of water between cool and warm halves of experimental apples; B, difference in % of water between halves of control apples.

naturally between stem and flower ends of the fruit, some had been placed in the apparatus stem up, others stem end down, and others with the stem-flower axis horizontal. Table 1 summarizes the data obtained from plugs from treated and control apples. Those from apples given a gradient treatment showed a distribution of water which was lowest on the warm side, highest in the middle, and next highest on the cool side. Statistical treatment indicated that the differences in percentage of water between warm and cool and between warm and middle samples were significant but that the difference between cool and middle sections was not. Since the highest percentage of

TABLE 1

DISTRIBUTION OF WATER AFTER 84-HOUR GRADIENT TREATMENT. AIR TEMPERATURE ON WARM SIDE, 20°C .; ON COOL SIDE, 6°C . (TESTED BY PLUG METHOD)

	Experimental	Control*
Number of samples.	20	6
Water % fresh wt. cool side.	85.56	84.53
Water % fresh wt. middle.	85.84	84.94
Water % fresh wt. warm side.	84.36	84.18
Cool minus warm.	1.20	0.35
Middle minus warm.	1.48	0.76
Middle minus cool.	0.28	0.41

* For controls both sides were at same temperature but one side was marked a, the other b. Differences are given as a minus b, middle minus b, and middle minus a.

wrinkling of the warmer sides; the cooler sides were somewhat more turgid than the controls. There was a small but measurable difference in percentage of water between the warmer and cooler sides of the fruits, but the data were not clearly significant when treated statistically. The differences noted, in this case, may have resulted from the warm side losing more water by evaporation. Microscopic observations of freehand sections of the succulent tissue of these young fruits showed only occasional intercellular spaces. When portions of the fruits were placed in a dilute solution of India ink in a suction flask which was

evacuated during 30 minutes, there was almost no penetration of the ink into the tissue except near the edges where the cells had been torn in the cutting.

Fruits on the tree are subjected to a marked temperature gradient only during the time that the sun strikes their unshaded side. An attempt to duplicate the effect of such an alternating gradient is shown graphically in figure 3. Experimental apples were given a 12-hour gradient treatment (cool side 6° C., warm side 35° C.) and were then freely sus-

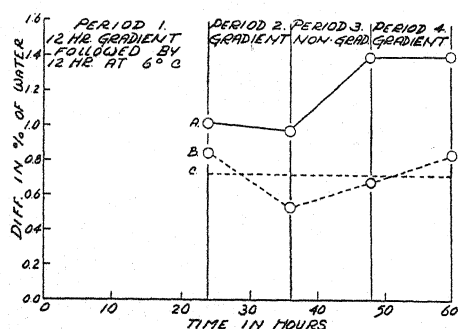


FIG. 3.—Movement of water in apples during alternating periods of gradient and nongradient treatment. A, difference in % of water between cool and warm halves; B, difference in % of water between stem and floral halves of controls; C, arithmetic average of curve B.

pended in the cold room for 12 hours. This alternating treatment was carried out for 2½ days, with samples taken at the end of each 12-hour period after the first 24 hours. Controls were kept both in the incubator and in the cold room.

In figure 3 the small movement of water from warm to cool side in period 1 and the apparent reverse movement during period 2 may be accounted for by excessive evaporation from the fruits during the early part of the treatment. This makes it difficult to draw conclusions; however, a comparison of curve A with B or C indicates that there was movement of water from the warmer to the cooler side of the experimental fruits

during the gradient treatment and during nongradient (period 3). The latter is explained by the fact that after removal from the gradient treatment there is a period of time before the fruit comes to a uniformly constant temperature, and water may continue to move during that time.

Thermocouples were used to measure the differences in temperature between the shaded and sunny sides of Wealthy apples naturally attached to the tree. Temperature readings were taken near noon on almost cloudless days in August when the air temperature was 28°–32° C.

TABLE 2
LARGEST TEMPERATURE DIFFERENCES BETWEEN TWO SIDES OF WEALTHY APPLES DURING 1-HOUR TEST PERIOD UNDER NATURAL CONDITIONS (AIR TEMPERATURE 28°–32° C.)

Shady Side	Sunny Side	Gradient
30.0° C.	39.5° C.	9.5° C.
30.6	40.1	9.5
31.1	40.8	9.7

Table 2 gives the largest differences found during a 1-hour test period, showing that under natural conditions a 9.7° C. temperature gradient may occur. CURTIS (3) showed that, if such a gradient is maintained, water will move from the warmer to the cooler side of the fruit.

Discussion

The decrease in rate of movement of water across the apples, after the first 3 days of gradient treatment, probably resulted from the water being held more strongly by the osmotic and imbibitional forces of the desiccated side. In young apples the small amount of water movement may be explained on the basis of their lack of intercellular spaces, which limits the chances for movement of water by distillation; it is possible that the

water moved by capillarity through the hydrophylic colloids of the cell walls.

In a natural situation one would expect to find an alternating temperature gradient. When the sun strikes a fruit only on one side, that side may become from 10° to 18° F. warmer than the shaded side. When a fruit is removed from such a situation, it is reasonable to assume that there will be a period of time before the fruit comes to a uniformly constant temperature. During that time water will continue to move from the warmer to the cooler side. After the fruit has reached a uniformly constant temperature, the imbibitional and osmotic forces of the more desiccated side are not strong enough to reverse the process to any extent.

Summary

1. Mature apples (variety Wealthy) exposed to a temperature gradient show a fairly rapid rate of water movement across the fruit (from the warmer to the cooler side) for the first 3 days and then a slower rate of movement. Gradient treat-

ment of apples caused significant amounts of water to move across the fruits in less than 48 hours.

2. After a $3\frac{1}{2}$ -day gradient treatment plugs from mature apples showed a greater percentage of water in the middle, next highest on the cool side, and lowest on the warm side. The controls also showed a higher percentage of water in the middle.

3. After 3 days of treatment a small but measurable difference in percentage of water was found between the warm and cooler sides of young ($1-1\frac{7}{8}$ inch diameter) apples.

4. Mature apples subjected to alternating 12-hour gradient and nongradient treatments showed water movement from the warmer to the cooler sides.

5. Wealthy apples on the tree, during a sunny summer day, showed a maximum temperature gradient of 9.7° C. between the sunny and the shaded sides.

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RELATIONSHIPS OF AMPHOLYTES TO ASSIMILATION AND RECOVERY OF AMMONIUM AND NITRATE NITROGEN IN PLANT TISSUE

V. A. TIEDJENS

Introduction

In experiments on nitrate and ammonium assimilation (20, 21, 22), the determination of the respective ions in plant tissue resulted in values which indicated that they were not being satisfactorily recovered by the accepted methods (18). This was particularly true for plants grown wholly or in part with ammonium nitrogen as the nitrogen source.

In a previous paper (20) the term "combined ammonia" was used to designate a nitrogenous fraction which was recovered by aspiration with sodium hydroxide. This was an ammonium ion, which was slowly liberated after the so-called free ammonium ions had been removed with a weak alkali (sodium carbonate). In tomato plants grown with nitrate nitrogen this fraction was usually negligible, but in those grown with ammonium nitrogen (22) it was particularly large. No explanation has been given for this phenomenon. The possibility that the "combined ammonia" originated as a disintegration product of glutamines is not borne out by the data. They show that, in addition to "combined ammonia," there was present also a "combined nitrate" fraction which could only be recovered by taking cognizance of LOEB's theory (5) of ion displacement in a colloidal system; that is, the ammonium and nitrate ions apparently formed weakly dissociated salts with soluble and insoluble organic nitrogenous

compounds (ampholytes) which were present in the aqueous extract of tomato-plant tissue as well as in the protein-free extract. That this is possible has been shown by ROBBINS (17). The hydrogen-ion concentration of the nutrient solution supplied to plants in sand culture and differences in pH of the extracting water in the tissue analysis caused variations in the amounts of both nitrate and ammonium ions that were recovered.

When an aliquot of extract, from plants grown with ammonium nitrogen and from which the coagulable protein had been removed, was aspirated for ammonium or nitrate nitrogen, the ammonium ion was extracted only after 12 or more hours of aspiration instead of the 2- to 3-hour period as recommended (18). When a known quantity of a pure ammonium salt was aspirated with a mild alkali (sodium carbonate), the ammonium ion was completely recovered in 2-3 hours. However, the aspiration of aliquots of protein-free extract from plants grown with ammonium nitrogen required 30 or more hours before the last traces of ammonium nitrogen were displaced. Tomato plants grown with nitrate nitrogen usually have so little ammonium present (20) that the slow recovery observed above is easily overlooked. There is thus considerable evidence that amino acids (glycine found in plants by PEARSALL and EWING [14]) and proteins in protoplasm could react with ions absorbed from a nutrient solution.

That this occurs in plant tissue by virtue of the amphoteric nature of constituents in the protoplasm was suggested by ROBBINS (17).

Investigation

Data are presented which show the amphoteric properties of amino acids, proteins, and other plant materials (table 1). Whether nitrate or ammonium

nitrate ions, liberating ammonium ions, with equilibrium established at a less acid pH. Glycine has an isoelectric point at pH 6.1 and reacts in a similar manner to asparagine above and below this point. Data from pure casein (fig. 1) also show how an amphoteric substance responds at different pH values. Thus it is probable that many substances found in protoplasm, and with widely different

TABLE 1
CHANGES IN PH AFTER ADDITION OF AMPHOLYTES TO 50-ML. STOCK SOLUTIONS OF
AMMONIUM NITRATE PREVIOUSLY ADJUSTED TO INDICATED PH VALUES
WITH NITRIC ACID OR AMMONIUM HYDROXIDE

INITIAL PH OF STOCK SOLUTION	KIND AND AMOUNT OF AMPHOLYTE ADDED					
	Asparagine, 0.5 gm.	Glycine, 0.5 gm.	Casein isoelectin, 3.5 gm.*	Pectin commercial, 0.5 gm.	Calcium pectate, 0.5 gm.	Soybean protein, 2.0 gm.
1.9.....		+2.1	+1.5	+1.4	+1.3	+1.9
2.2.....	+1.2		+1.9	+1.1	+1.6	+2.2
2.8.....		+1.2	+1.8	+0.7	+1.9	+2.2
3.0.....		+1.3	+1.7	+0.6	+2.2	+2.2
3.2.....		+1.2	+1.6	+0.4	+2.8	+2.2
3.5.....	+0.5		+1.4	0.0	+3.0	+1.9
4.0.....	0.0	+1.1	+0.8	-0.4	+2.8	+1.6
5.6.....	-1.6	+0.2	-0.6	-2.1	+1.2	0.0
6.8.....		-0.5	-1.6	-3.6	+0.3	-1.2
7.6.....	-3.3		-2.2	-4.1	-0.1	-1.6
8.3.....		-1.1	-2.3	-4.7	-0.4	-2.1
8.9.....	-2.5	-1.3	-2.8	-5.1	-0.3	-2.7
9.6.....		-1.4	-3.1	-5.8	-0.2	-2.8
10.2.....	-2.4		-3.0	-5.4	-0.1	-2.0
10.3.....		-1.6	-2.4			-0.2
Exchange neutrality (pH).....	4.0	6.1	4.7	3.5	7.4	5.6

* Stock solution of casein isoelectin = 100 ml.

ions from ammonium nitrate combine with these materials depends on the pH of the solution in which they are dispersed or dissolved, as ROBBINS (17) has pointed out. Above pH 4.0, asparagine combined with ammonium ions, liberating nitrate ions and causing equilibrium in the solution to be established at a lower pH. In a solution more acid than pH 4.0, asparagine combined with the

isoelectric points, have these amphoteric properties.

If ammonium and nitrate ions form dissociation compounds with ampholytes in the cell, theoretically it should be possible to displace different amounts of the ions from plant tissue by extracting it with water at different pH values. That this is possible is shown by the data in figure 2. They were obtained by placing

100-gm. aliquots of ground tomato tissue, from plants grown in soil, in 800 ml. of distilled water and adjusting with HCl or NaOH to the indicated pH values. After 30 minutes the liquid was pressed out as much as possible, the residue being washed twice with 100 ml. of water. Soluble proteins were not coagulated. The

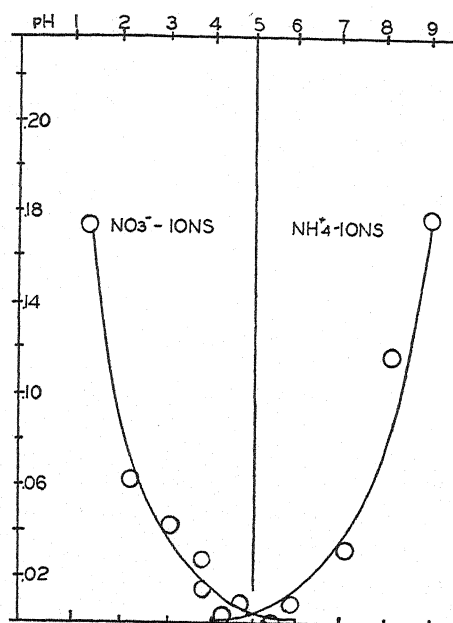


FIG. 1.—Absorption of nitrate and ammonium ions by casein in solutions of ammonium nitrate adjusted to different pH values. Figures on left are gm. of ions in 100 gm. dry matter absorbed by casein. Intersection of curves is at isoelectric point of casein.

data show that, as the pH is lowered to 3.0, less and less nitrate is extracted (less is dissociated from ampholytes). Much larger quantities were extracted at pH 10. On the other hand, less ammonium nitrogen was recovered as the pH value was increased. In other words, with change in pH either the ammonium or the nitrate ions apparently become dissociated from the amphoteric substances in the tissue so that there is no one pH value at which all the nitrate or ammonium ions will be easily recovered. In

this particular experiment, when tissue was extracted in distilled water at pH 5.4 (unadjusted), less than half the nitrate but at least 75% of the ammonium nitrogen was recovered. That the remaining nitrate and ammonium nitrogen was combined with the coagulable protein or amino acids is shown by a comparison of two methods of extraction—namely, aqueous extraction and electrodialysis—of young green tomato stems which had been ground and divided into three 100-gm. aliquots. The data (table 2) show

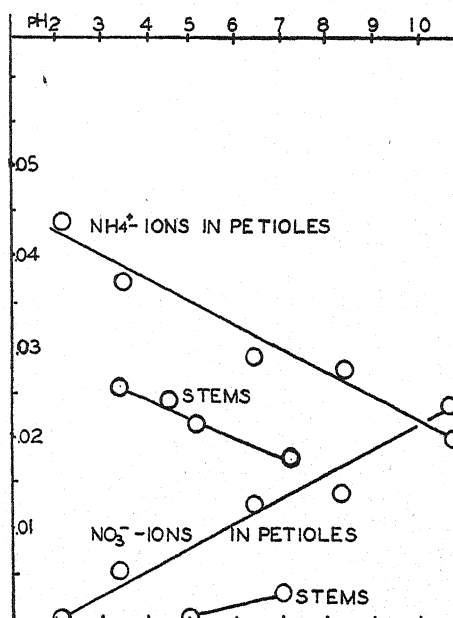


FIG. 2.—Nitrate and ammonium ions extracted from young tomato plant tissue in 800 ml. of water for 30 minutes at indicated pH values. Figures on left represent gm. of nitrate or ammonium nitrogen removed from 100 gm. of green tissue.

that more nitrate and ammonium ions were removed by electrodialysis than by aqueous extraction and, furthermore, that the coagulated protein removed from the aqueous extract contained some nitrate but no ammonium nitrogen.

Three groups of tomato plants were grown in soil adjusted to pH 4.0, 6.0, and

7.4. Each group of plants was divided into three lots which were supplied, respectively, with nutrient solutions containing ammonium sulfate, sodium nitrate, or ammonium hydroxide. Samples of leaf tissue were removed when the plants were 10-12 inches tall and were analyzed by electro dialysis as well as by aqueous

extraction. For plants supplied with nitrate nitrogen there was very little difference in the amounts of ions recovered by the two methods of extraction (fig. 3). When grown with ammonium sulfate, the method of extraction affected the recovery of ammonium nitrogen from plants grown at pH 4.0 but not from those grown at pH 6.0 and 7.4. This may be explained by the differences in growth and in the rate of nitrogen assimilation in plants rooted in soils of different pH values (22). The nitrate nitrogen recovered by the two methods of extraction showed large differences in plants grown with either ammonium sulfate or ammonium hydroxide.

Tomato plants were grown in sand cultures at different pH values maintained by continuously flowing nutrient solution. The data shown in figure 4 were obtained from plants supplied with calcium nitrate as the source of nitrogen. The recovery of ammonium nitrogen showed little difference between the two methods of extraction, but the method

TABLE 2
NITRATE AND AMMONIUM IONS EXTRACTED
FROM TOMATO-STEM TISSUE BY
VARIOUS METHODS

SAMPLE	METHOD OF EXTRACTION	MGM. IN 100 GM. GREEN TISSUE	
		Nitrate	Ammonium
1.....	Electrodialyzed	510	49
2a.....	Aqueous extraction	347	27
2b.....	Coagulated proteins from 2a	14	0
3.....	Aqueous extraction
3a.....	Electrodialyzed before removal of proteins	497	51
3b.....	Electrodialyzed after removal of proteins	354	34

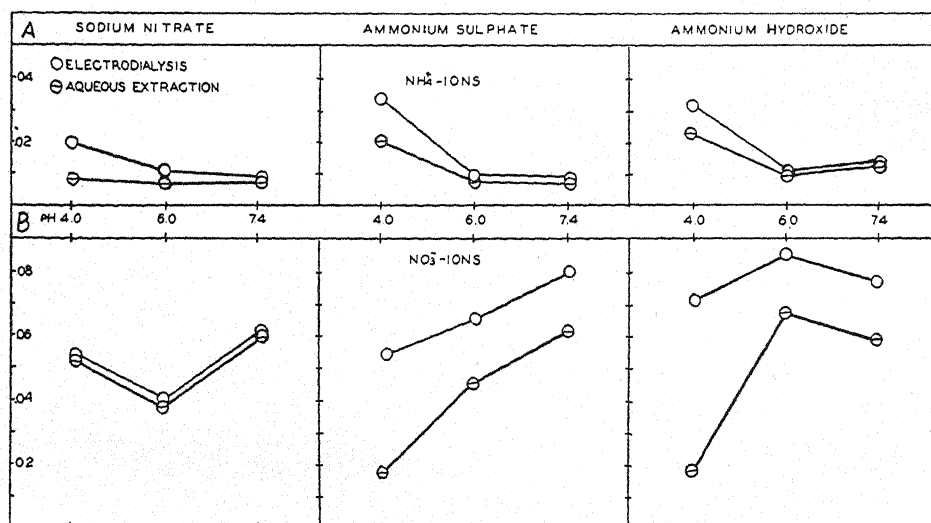


FIG. 3.—Nitrate and ammonium ions recovered from tomato-plant tissue by two methods of extraction from plants grown on soils having three different pH values. Figures on left represent gm. of nitrate or ammonium ions in extracts from 100 gm. of green tissue.

used had considerable influence on the amount of nitrate nitrogen recovered from plants grown at pH 3.5, 4.5, and 7.5. Apparently nitrate ions were combined with ampholytes in the plants grown at pH 3.5, 4.5, and 7.5. It is interesting to note that the point of exchange neutrality¹ for tomato-stem tissue is be-

the natural pH of each extract. More nitrate nitrogen was extracted in each case by electro dialysis than by aqueous extraction; the differences were 119% in the roots, 126% in the stems, 43% in the petioles, and 126% in the leaf blades. The total amount of nitrate varied; it was highest in the roots and least in the blades. The percentage amount of combined nitrate is correlated with the comparative amount of protein nitrogen in the roots, stems, and leaves of tomatoes grown with sodium nitrate, as shown graphically in figure 2 of an earlier publi-

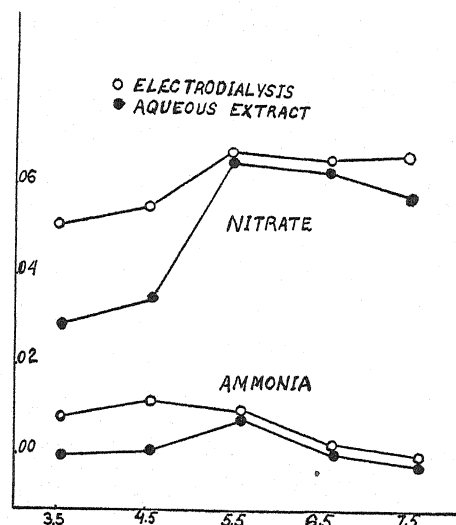


FIG. 4.—Amounts of nitrate and ammonium nitrogen recovered by two methods of extraction of tomato plant tissue. Plants grown in sand with calcium nitrate as source of nitrogen. Scale represents gm. in 100 gm. of green tissue.

tween pH 5.5 and 6.5. It would seem that nitrate ions can be easily recovered from such tissue by aqueous extraction between these points.

In figure 5 are shown similar data for roots, stems, petioles, and leaf blades of tomato plants grown in sand culture with nitrate nitrogen. Extraction of samples representing the four regions was carried out under uniform conditions; no attempt was made to change

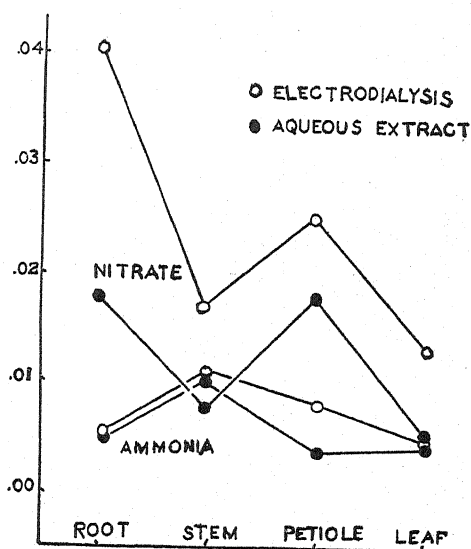


FIG. 5.—Amounts of nitrate and ammonium nitrogen removed by electro dialysis and aqueous extraction from different tissues of tomato plants grown in sand culture with no attempt to control pH of nutrient solution. Scale represents gm. in 100 gm. of green tissue.

cation (22). In leaves, with five times as much protein nitrogen as in roots, there was 126% more nitrate recovered by electro dialysis than by aqueous extraction. The roots and stems had approximately the same percentage content of protein, but the percentage increase in nitrate nitrogen extracted by electro-

¹ The point of exchange neutrality is used to designate that pH value at which the free ampholyte combines with an equal number of cations and anions in a particular salt solution in exchange for hydrogen and hydroxyl ions.

dialysis over aqueous extraction was slightly higher in the stems than in the roots. There is considerable difference between fine-root and stem tissues in the types of proteins and amino acids found.

Discussion²

MICHAELIS (8) has shown that casein and gelatin have isoelectric points at approximately pH 4.7 and that these proteins form dissociable compounds with anions in a solution having a pH value above the isoelectric point. MATTSON (7) carried this work further and found that the laws governing the soil ampholytoids—that is, the chemical behavior of the “silicated, phosphated and humated compounds of the sesquioxides”—may be extended to proteins in the soil which are in effect derived from plants and are part of the organic matter of the soil. Proteins, then, when in the presence of anions and cations at any given pH, will form compounds which dissociate either as anions or cations, depending on their isoelectric points.

There is some evidence that other than nitrate and ammonium ions are combined in plant cells. LUTEMAN and WALBRIDGE (6) suggested that magnesium is found in combined and uncombined forms. Whether the combined magnesium is anything more than that present in the chlorophyll molecule is not clear; since metallic ions do associate with organic matter in the soil (7), we may assume that they also do so in protoplasm. NIGHTINGALE *et al.* (9) have shown that calcium may be combined with the protein complex and that, when

calcium-deficient plants, which have stopped growing because of a lack of soluble calcium, are placed in continuous darkness, proteolysis releases a sufficient amount of usable calcium so that growth may be resumed. NIGHTINGALE *et al.* (12) suggested the possibility that potassium may be held by protein, although they recovered practically all the potassium by water extraction. This, however, does not prove that potassium may not be so combined. They (13) found no release of sulfate ions when the residue from water extraction was electrodialedyzed. Here again the hypothesis that ions combined with ampholytes was not disproved.

In view of the fact that the data show a chemical combination between the ampholytes of the cell and the absorbed common ions or electrolytes (nitrate and ammonium ions), it is of interest to know whether the relationship of this phenomenon to the assimilation of nitrate and ammonium ions is more than a buffer system in the plant and whether it may account for observed phenomena in the determination of ammonium and nitrate. The following observations indicate the existence of such dissociation complexes in tissue extracts: (a) Electrodialysis in many cases recovers more anions and cations than is possible by aqueous extraction (figs. 2, 3). (b) The quantity of nitrate and ammonium ions recovered by aqueous extraction of tissue depends on the pH of the extracting fluid (fig. 2). (c) The recovery of ammonium ions from aqueous extraction by aspiration with weak alkali is a prolonged process compared with recovery from a pure ammonium salt. (d) The aspiration method of SESSIONS and SHIVE (18), using sodium carbonate for ammonium and sodium hydroxide for nitrates, recovers additional ammonium ions with sodium hydrox-

² In this discussion only nitrate and ammonium ions are used for illustrating certain reactions. Other anions and cations would, of course, be present and would complicate the reactions in the cell. Whether other anions and cations behave in a similar manner requires further investigation. Preliminary indications are that they do.

ide which apparently are not derived from the reduction of nitrate ions.

These observations would indicate that the chemical attraction between ampholytes and the common ions not only is a phenomenon characteristic of the proteins, as suggested by THERON (19) and LOEB (5), but seems associated

MICHAELIS (8) listed a number of amino acids which occur in plants; their isoelectric points range from pH 2.76 for aspartic acid to pH 10.97 for arginine. Some of these are placed on the graph in figure 6, showing the theoretical curves for ammonium and nitrate absorption and accumulation in growth processes.

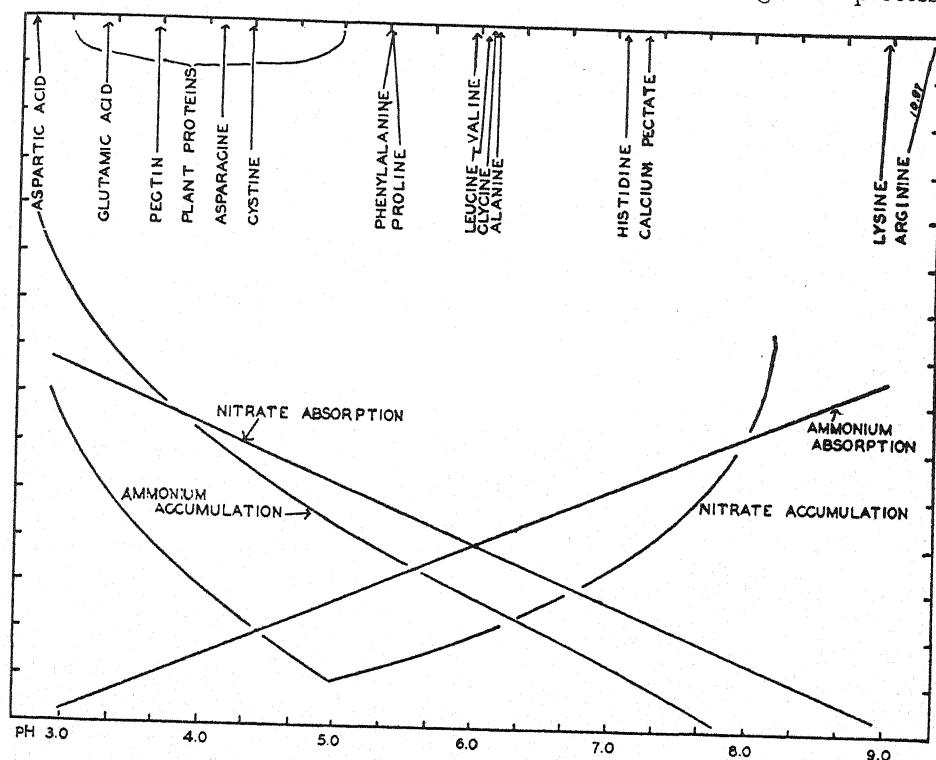
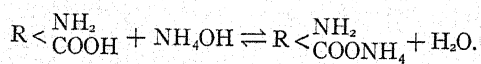
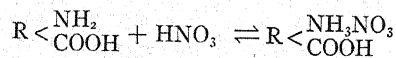


FIG. 6.—Distribution of amino acids on pH scale according to isoelectric points, and theoretical curves showing absorption and accumulation of nitrate and ammonium nitrogen in plant tissue adjusted to different pH levels. It shows possible role that amino acids play in adsorption of nitrate and ammonium nitrogen. At points more acid than their isoelectric points they adsorb nitrates and at points more alkaline they adsorb ammonia. Scale represents gm. per 100 gm. of dry matter.

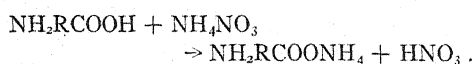
with the soluble ampholytes as well. A mechanism has been suggested by LOEB (5) which shows how these ions may be combined with protein:



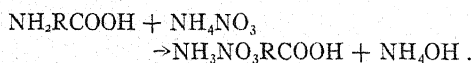
If aspartic acid (pH 2.76) and asparagine (pH 4.1), isoelectric below pH 4.2, exist as free ampholytes in the cell, they would combine primarily with ammonium ions (or cations in general) at any pH of the sap above their isoelectric points. The hydrogen of the ampholyte would be displaced by the ammonium ion. Arginine (pH 10.97), lysine (pH 9.4), and

histidine (pH 7.15)—amino acids which PEARSALL and EWING (14) reported as being present in the potato—have isoelectric points above 7.0 and would combine primarily with nitrate ions and with anions in general if the pH of the cell sap was more acid than pH 7.0, although histidine may combine with cations in rapidly dividing parenchymatous cells which ECKERSON (2) stated may have a pH of 7.4.

The following reactions may take place: Suppose asparagine and aspartic or glutamic acids, with isoelectric points lower than pH 4.2, occur in tomato sap at pH 5.4. They would dissociate hydrogen ions, whereas dissociation of the hydroxyl ions would be suppressed.³ They would react with ammonium nitrate as follows:



The ammonium ion would form a weakly dissociated salt with the ampholyte. The sap would tend to become more acid because of the free nitric acid. If histidine, lysine, and arginine, with isoelectric points above pH 7.0, also occur in the tomato juice, they would dissociate hydroxyl ions, while dissociation of hydrogen ions would be suppressed. They would react with ammonium nitrate as follows:



The nitrate ions would form weakly dissociated compounds with the ampholytes. The sap would tend to become more alkaline. The free acid and am-

monium hydroxide would neutralize each other, and any change in reaction would depend on the amount of ampholytic material having isoelectric points at different pH values.

The process—ampholytic behavior of protoplasm—in the plant cell, however, is not so simple as these reactions would indicate. A large number of amino acids having isoelectric points between pH 5.0 and 6.5—such as phenylalanine, cystine, tyrosine, valine, leucine, alanine, and proline—would dissociate hydrogen, hydroxyl, or protein (ampholyte) ions, depending on the pH of the sap of any particular tissue. These ions would enter into chemical combination with electrolytes and form dissociable compounds. The pH values of the isoelectric points of these amino acids are so close to the pH values of sap in many cells that they might change their charge several times during a day since, according to INGALLS and SHIVE (4), the composite pH of cell sap fluctuates considerably.

The data suggest that three types of nitrate and ammonium ions are present in plants which are absorbing these ions from a nutrient solution: (a) free ions in solution as mineral salts; (b) ions associated with amino acids and other soluble plant products which may be quite mobile; and (c) ions associated with proteins and other materials which are in a more or less colloidal condition or may even be insoluble and become part of the reserve material in the plant. If this assumption is correct, it offers a possible explanation why a DONNAN equilibrium hypothesis (1) is difficult to apply to data when the ion concentration in the plant is much higher than that existing in the nutrient solution. The concentration of any given free ion in an absorbing cell is probably not higher than the concentration in the nutrient solution surrounding

³ The papers by FLINT (3) are extremely interesting regarding H^+ and OH^- ions. The validity of his hypothesis is recognized. H^+ and OH^- ions are used for positive and negative charges in the present paper.

it. Chemical data which show a higher concentration of nitrate in a composite sample of tissues than is present in the solution surrounding the roots does not prove that a DONNAN equilibrium does not exist in the root hairs. The higher concentration in the composite sample may be accounted for by the ions associated with soluble or insoluble organic materials in cells other than those that actually absorb the nutrients. Furthermore, it is difficult to interpret data obtained from a mass of plant tissue in terms of the concentration in the nutrient solution. The DONNAN equilibrium hypothesis must be applied to those root-hair cells where absorption takes place and not to those cells much farther removed from the absorbing region. In tomato, in which nitrate occurs throughout the plant, much higher concentrations are recorded (11) than in apple (21) or asparagus (10), in which it is seldom found except in the fine roots. It is probably safe to assume that a DONNAN equilibrium does exist with respect to cells instrumental in absorbing ions from the nutrient solution.

On the basis of the data presented it would seem that a logical explanation for the slow recovery of ammonium and nitrate ions from plant extracts containing those ions is available. During the aspiration for the recovery of ammonium ions, the pH remains above 10, establishing negative charges on most of the amino acids present. These charges would readily be neutralized by some ammonium ions and other cations. Ammonium ions which are combined as mineral salts would be easily removed by aspiration, but those associated with soluble ampholytes would not be removed any faster than equilibrium could be re-established through replacement of ammonium by sodium ions after the free

ions were removed. This would be a slow process.

The determination of nitrate nitrogen should be a more rapid process if the resulting ammonium ions were removed as soon as they are formed, because most of the nitrate ions probably exist as free or inorganic-salt ions. However, the aspiration process usually is much slower, so that equilibrium is established between the ammonium ions reduced from nitrate and the soluble ampholytes before the ammonium ions are removed from the solution. The determination of nitrate would then involve the same association and dissociation phenomena attendant to the determination of ammonium nitrogen. The greatest difference in the amounts of ions obtained by extraction with water and by electro dialysis occurs at those pH values farthest from the isoelectric points; the least difference is found when the greatest volume of ampholytic material is at its isoelectric point. In the case of tomato tissue the latter is around pH 6.0 to 6.5.

The explanation, therefore, for the retention of ions in cells in higher concentrations than that present in the nutrient solution seems comparatively simple. If we assume that the pH of the protoplasm of any given cell is the algebraic sum of all positive and negative charges where mineral anions and cations are in equilibrium with one another as well as with soluble organic anions and cations or with dissociated charges on cell colloids, a further assumption is necessary to explain the change in acidity of a nutrient solution as ions are absorbed. On the basis of the DONNAN equilibrium hypothesis, we may assume that there is a continual exchange of free ions through the cell membrane. The nitrate and ammonium ions must then play a role in changing the equilibrium in the cell by

means of the chemical changes attendant to the formation of amino acids with soluble carbohydrates, because it has been shown that changes occur in the equilibrium of the nutrient solution only when nitrate and ammonium ions are being assimilated (22). NIGHTINGALE (by letter) suggested that changes in pH occur at the surface of the cell membrane, even though a constant pH is maintained in the nutrient solution. In other words, if nitrate or ammonium ions are being rapidly assimilated (amino acids synthesized), the ions with which they were associated remain at the cell membrane and cause a temporary change in acidity, the degree depending on the rapidity with which excreted ions diffuse and come into equilibrium with the other ions in the solution. In water cultures this would be much more rapid than in sand cultures, which, in turn, would facilitate more rapid diffusion than in a soil. The fact that the ions associated with the nitrate or ammonium ions do not enter the cell as rapidly seems indirect proof that a DONNAN equilibrium phenomenon does exist—the concentration of free plus dissociable ions in the cell probably is never greater than in the nutrient solution.

It would seem that it is necessary to attribute another function to absorbed ions. From the results obtained by MATTSON (7) and evidence by the above data, certain ions have the function of keeping the protoplasm in a hydrated or highly dispersed condition. Cells of meristematic root tissue have a dense, hyaline protoplasm more or less uniform in appearance, containing the nucleus and occasionally other bodies of greater density. Proteins, which are insoluble organic complexes at their isoelectric points, and abundant in rapidly dividing cells, seem to be hyaline in appearance except when they are colored by pig-

ments. In rapidly dividing cells they are probably in a highly dispersed condition.

ECKERSON (2) stated that parenchymatous cells of this nature have pH values as high as 7.6. Casein and soybean-seed protein become so highly dispersed in the presence of ammonium hydroxide at pH values above 6.5 that they cannot be separated from water with ordinary filters. The same proteins flocculate at pH 4.6 to 4.7—the pH of their isoelectric points—and are then readily separated from water by filtration. PEARSALL and EWING (14) stated that many vegetable proteins have isoelectric points between pH 3.0 and 5.6. ECKERSON (2) reported that xylem tissue is acid (pH 4.4) and that mature cells in which the protein may be precipitated are acid. In other words, the proteins are near the pH values of their isoelectric points and are no longer dispersed in the cells because of the acid reaction.

Cells which are primarily concerned in the assimilation of nitrate and ammonium ions, therefore, have saps with comparatively high pH values and viscous, highly dispersed protoplasts. The abundant protoplasm in the cells of phloem, cambium, other meristems, parenchyma, and root hairs consists of highly dispersed proteins in which there are many soluble amino acids, electrolytes, sugars, fatty materials, and other substances, some of which have amphoteric properties. One would expect a high degree of association between the ammonium and nitrate ions and the proteins in such a highly dispersed system.

When plants become "hard"—high in carbohydrates—many of these hyaline, rapidly dividing cells mature very quickly, and the composite pH of the tissue therefore becomes more acid (2), probably because of the presence of organic acids. The proteins become precipitated

and give the protoplasts a stringy-granulated appearance. It has been shown that tomato plants in this "hard" condition become very succulent soon after a nutrient solution containing ammonium is supplied, much more so than when nitrate is supplied (20). This would seem to be strong evidence that organic acids combine with the absorbed ammonium ions (a slower process with nitrate ions because they must first be changed to ammonium). The pH values of the cells are raised, causing the protoplasts to become dispersed and amino acids to be formed. This is soon followed by cell division, and evidence of growth is seen.

The dispersion of the proteins, therefore, probably results from combination with cations absorbed from the nutrient solutions. It has been shown (23) that sodium is very efficient in increasing the water-holding capacity of plant tissue and that calcium tends to counteract the effect of sodium. Many of these cations probably do not exist as free ions, as suggested by HOAGLAND, quoted by THERON (19), but as dissociation products of colloidal compounds (proteins) and amino acids. The degree of dispersion in a cell with a sap at pH 7.0 is then the result of the ratio of the various cations. MATTSON (7) also has shown that calcium suppresses the dispersion, particularly as the majority of the proteins may be considered electronegative. MATTSON further showed that iron and aluminum precipitate proteins and form undissociated complexes. These complexes have isoelectric points at pH values between the pH 4.0 of protein and pH 8.0 of the iron salt employed (in this case no aluminum was present in the nutrient solutions); the actual value depended on the proportion of protein to iron salt in combination. This may have some bearing on the difference in iron assimilation observed

between nitrate- and ammonium-supplied trees in an experiment in which the effect of pH on nitrate and ammonium assimilation (21) was studied.

The high degree of dispersion or swelling of proteins with cations indicates a negative charged colloid of high intensity. These proteins are then able to combine with amino acids and electrolytes. The complex formed would have negative and positive charges which would be exchanged for other anions and cations. The pH of a cell would then be the point at which equilibrium of the dissociating negative and positive charges was established. The absorption of nitrate and ammonium and other ions would depend on the number of negative and positive charges which could be displayed at any given pH. The number of free ions in the cell would depend on the concentration in the nutrient solution, while some are combined with ampholytes in the cell. The pH of a cell may then be considered as resulting from exchange reactions with reference to the absorption of anions and cations. That there would be considerable overlapping is graphically shown in figure 1. This overlapping of absorption of cations and anions is the result of the ratio of dissociation of negative and positive charges, depending on the relation of the pH to the nutrient solution and the pH of the isoelectric points of the various ampholytes present. Theoretically, cation or anion absorption would not cease until the pH of the nutrient solution was more acid or more alkaline than the pH of the lowest or highest isoelectric points of the constituent parts that make up the protoplast. Fortunately, these conditions seldom occur under normal conditions, because the protein would become coagulated or so highly dispersed that life could not exist. PIRSCHLE (15), using nitrate nitrogen, obtained a bimodal

curve for growth of some plants over the pH range from 3.0 to 9.0. He found less vigorous growth in many plants at pH 6.0 than at pH 4.0 or 8.0. This may have been related to the ampholytic nature of protoplasm.

RABER (16) assumed that the so-called "cell membrane" is negatively charged. The cell membrane of an absorbing cell, however, is probably in equilibrium with its protoplast and may carry negative or positive charges, depending on the pH of the nutrient solution and upon the nature of the material at the periphery of the cell. If the membrane or material has amphoteric properties, it probably carries either one or the other charge, depending on the relation between the pH of the isoelectric point and the pH of the nutrient solution. This would seem reasonable, in view of the fact that both ions must be absorbed in order to account for the growth PIRSCHLE obtained at different pH values. It must be remembered that the absorbing area of feeding roots is the newly formed region back of the tip where root hairs are abundant. Any effect of a nutrient solution on the assimilation of ammonium or nitrate ions is exerted through these root hairs, especially in the apple, in which the assimilation of nitrate ions to amino acids occurs in the very fine roots (21).

The synthesis of amino acids in the roots of apple, accompanied by utilization of carbohydrates, undoubtedly causes changes in the pH of the cell contents, which helps to account for the diurnal variations, observed by INGALLS and SHIVE (4), in the pH of the composite plant tissue. THERON (19) has shown that the pH of the nutrient solution also affects the buffer capacity of the protoplasts of the root cells quite materially. These changes in the pH of the cells therefore account for a system that

is shifting back and forth, in which the ratio of cations to anions that displace positive and negative charges is continually changing. This change in ratio of charges and the effect of the anions and cations thus combined on the dispersion of the protoplast and the saponification of fats undoubtedly influence markedly the growth and division of cells and the assimilation of ammonium and nitrate ions. These processes are probably most favored just above or below the normal pH of the cell sap. The rate at which the two ions are assimilated then depends on the reduction of the nitrate ions. If more ammonium ions are being absorbed by one plant than are made available by the reduction of nitrate ions in another plant, amino acids will accumulate more rapidly in the former, providing, of course, that carbohydrates are plentiful.

From this discussion it may be assumed that any method of removing anions or cations from living tissues which does not take into consideration the isoelectric points of proteins and amino acids would fall far short of giving more than comparative data. For complete recovery the ions must be disposed of by electrical devices which remove them as soon as they are released through equilibrium phenomena, or the tissue must be completely destroyed so that the ions are released to a free state. This discussion may throw some light on the difficulties encountered in obtaining data which agree or check according to theoretical concepts.

Summary

Data are presented which show that nitrate and ammonium ions may not be recovered from living tissue in absolute amounts. This results from the fact that protoplasm contains proteins and amino

acids which have amphoteric properties and which form dissociation compounds with nitrate and ammonium ions which obey the laws of electrochemistry. The conditions in cells necessary to support the above assumption are suggested, to-

gether with an explanation of conditions which favor nitrogen assimilation and subsequent growth in plants.

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PIGMENT GLANDS OF COTTONSEED. III. DISTRIBUTION AND SOME PROPERTIES OF COTTONSEED PIGMENTS

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Introduction

For a number of years the direct solvent extraction of cottonseed for the production of oil and meal has been recommended as a practical method of processing (9, 10, 11, 13, 14, 19, 20, 24, 25), but it has only recently been applied on a commercial scale in the United States. The recent successful development of solvent extraction methods for the processing of soybeans (12), as well as the current trend toward the use of processes requiring the employment of a minimum of labor, has intensified interest in the application of this method to cottonseed. However, the unique system of pigments in the kernel of the cottonseed not only differentiates this seed from others but poses problems which are not encountered in the processing of other commercial oilseeds. Although the control of color is not the only difficulty, it is one of the principal ones encountered in application of solvent extraction to cottonseed on an industrial scale.

It has been shown (4) that the pigment glands, which contain most of the pigments of the kernel, are mechanically very strong and are resistant to the action of a considerable number of types of organic solvents such as the hydrocarbons, chlorinated hydrocarbons, and glycerides. These glands possess a density of approximately 1.378 gm./cc., which is less than that of the other tissue of this seed. On the basis of these proper-

ties a method which employs mechanical disintegration and flotation was devised as a means of separating pigment glands from other seed tissue and oil.

It has also been shown (6) that the pigment glands owe their mechanical strength to a thick encompassing wall, composed of cellulose impregnated with a pectinaceous material and exteriorly coated with a thick layer of cutin. This wall is readily ruptured by contact with water and a few water-miscible alcohols, ketones, and ethers of low molecular weight. The relatively slow rupturing of the wall on contact with other organic liquids was found to result from the presence of moisture in the seed or solvent.

Even casual examination of a section of cottonseed will reveal that most of the deeply colored pigments of the kernel are concentrated in the pigment glands. The oil and extraglandular tissue are colored a faint yellow, whereas the glands may vary from yellow, through various shades of orange and red, to dark purple. It has generally been assumed that all the gossypol and most of the other pigments of the seed are segregated in the glands, but no accurate determination has heretofore been made of the extent to which this occurs or to differentiate the extra- from the intraglandular pigmented material. Consequently, the present investigation was undertaken with the object of ascertaining the distribution of the cottonseed pigments between the glands and the extraglandular tissue and oil. Further information has also been sought concerning the behavior of the pigments

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during extraction of the oil with different solvents.

Material and methods

PREPARATION OF SEED, MEAL, AND GLAND SAMPLES FOR EXTRACTION.—Samples of seed kernels were finely divided by flaking or grinding to insure subsequent contact of the pigment glands with the solvent. When small samples of seed were to be extracted, the kernels were ground in a Bauer mill or in a glass mortar and then passed through a U.S. No. 50 sieve. Larger samples were prepared by flaking the kernels on rolls set at 0.003 in. clearance; this produced flakes varying in thickness from 0.004 to 0.008 of an inch.

Samples of defatted meal were prepared in a Soxhlet apparatus by extracting weighed amounts of flaked kernels with petroleum naphtha² for 4–6 hours.

Samples of pigment glands and of gland-free meal were prepared by means of the previously described flotation process (4, 23). Some of the samples were freed of adhering tissue by prolonged agitation of flaked meats in the flotation mixture (4); others were freed of small residual amounts by shaking them with finely divided silica suspended in petroleum naphtha (23).

Samples of flaked or ground meats, of defatted meal, or of glands which had been wetted to produce preliminary rupturing were dried for 18 hours in a vacuum desiccator over anhydrous calcium sulfate before extraction with organic solvents.

PREPARATION OF EXTRACTS.—Small samples of seed, of defatted meal, or of pigment glands were extracted with chloroform, diethyl ether, or petroleum

naphtha under the conditions previously described (2, 5) as being essential to insure complete extraction and subsequent stability of the pigments; that is, by equilibration for periods of not less than 24 hours at 38° F., and in the absence of light. All extracts were stored under these same conditions, and their absorption spectra were determined within a period of 72 hours after the extraction was started. Extraction of pigments with aqueous solutions of water-miscible organic solvents was accomplished by equilibration for periods of 10–30 minutes.

Complete extraction of the residual gossypol from solvent-extracted meals, and from meals freed of pigment glands by the flotation method, was accomplished by the following adaptation of a recently published method (22) for the determination of gossypol in cottonseed and cooked cottonseed meal. To a weighed sample of meal, ground to pass a U.S. No. 50 sieve, there was added 30 ml. of 30% aqueous ethanol (by weight). The mixture of meal and solvent was shaken vigorously and allowed to stand for one-half hour. In order to dissolve the resultant suspension of gossypol, 70 ml. of 72% aqueous ethanol (by weight) was added. The mixture was then agitated and centrifuged. Alternatively, samples of gland-free meal were treated directly with 60% aqueous ethanol. Aliquots of the supernatant solution were transferred to chloroform for application of the alkaline extraction method (5) for the isolation of gossypol, followed by the antimony trichloride spectrophotometric method (3) for the determination of the concentration of the isolated gossypol.

The size of meal sample used in preparation of the aqueous ethanol extract was adjusted on the basis of its gossypol content. Since the gossypol content of meals

² The petroleum naphtha used in this investigation was a commercial pentane-hexane fraction, boiling range 95°–138° F.

extracted with petroleum naphtha varied from 0.5 to 2.0%, relatively small samples (0.25–1.0 gm.) of these meals were used. Larger samples (2.0–10.0 gm.) of diethyl ether-extracted and gland-free meals were used because of their low content of gossypol.

DETERMINATION OF ABSORPTION SPECTRA AND PIGMENT CONTENT.—All absorption spectra were measured with a Beckmann quartz spectrophotometer except those of the antimony trichloride reaction products, which were read in a Coleman monochromator spectrophotometer. Extinction coefficients of extracts were expressed as $E_{1\text{ cm.}}^{1\%}$ or as $E_{1\text{ cm.}}^{\text{gm./l.}}$ in terms of the weight of kernels, glands, or meals extracted. The extinction coefficients of extracted oils were expressed as $E_{1\text{ cm.}}^{1\%}$ in terms of the weight of solvent-free oil dissolved.

All absorption measurements were made on chloroform solutions to eliminate differences resulting from the effect of the solvent. The absorption spectra of extracts prepared with chloroform were read directly, those for solvent-free extracted oils after solution of the oils in chloroform, and those for extracts prepared with solvents other than chloroform after transfer to chloroform. In order to transfer the pigments from water-immiscible solvents to chloroform, aliquots of the extracts were evaporated under reduced pressure at room temperature, and the residues were dissolved in measured volumes of chloroform. When a water-miscible solvent was used for extraction, a measured volume of chloroform was added to an aliquot of the extract, after which water was added to the mixture until all the color had been transferred to the chloroform layer upon shaking and centrifuging the mixture.

The gossypol content of seed was determined by direct application of the

antimony trichloride spectrophotometric method (2) to chloroform solutions of the extracts. For the determination of the content of residual gossypol of solvent-extracted meals and of meals freed of pigment glands by the flotation process, gossypol was first isolated from the extracts by the alkaline extraction method (5); the antimony trichloride spectrophotometric method was applied to the isolated gossypol. Preliminary isolation of gossypol by the alkaline extraction method was also applied for the accurate determination of the gossypol content of petroleum naphtha-extracted oils.

The gossypurpurin content of seed, meal, and gland samples was estimated on the basis of the extinction coefficients of chloroform extracts at 564–567 μ , the wave-length region of maximum absorption of gossypurpurin (1, 5). The percentage concentration was calculated by means of the formula ($E_{1\text{ cm.}}^{1\%}$ extract/ $E_{1\text{ cm.}}^{1\%}$ gossypurpurin) $\times 100$.

Results

INTRAGLANDULAR PIGMENTS.—Anhydrous petroleum naphtha was used for extracting the oil and the oil-soluble extraglandular material from dried cottonseed. The absence of both gossypol and gossypurpurin was demonstrated by spectrophotometric examination of the extract; this constitutes evidence that all the gossypol and gossypurpurin of the seed kernel are segregated in the pigment glands.

Gossypol constituted from 39 to 49% and gossypurpurin from 0.61 to 1.73% of the weight of glands separated from several samples of pure-bred seed of different varieties of *Gossypium hirsutum* (table 1). Since all the gossypol and gossypurpurin was segregated in the glands, the gland content of the kernels was determined on the basis of the rela-

tive content of gossypol in the separated glands and in the corresponding kernels. The percentage, by weight of glands, in each sample was calculated by means of the formula: ($\%$ gossypol in seed/ $\%$ gossypol in glands) $\times 100$. Because of the instability of gossypurpurin in solution, similar calculations based on the ratio of this pigment in the seed and in the corresponding samples of glands were considered to furnish a less accurate measure of the content of glands. The content of glands in different samples of seed was found to vary within wide limits (2.37–4.81%).

The spectral absorption resulting from the presence of gossypol in the aforementioned samples of pigment glands (table 1) was calculated throughout the wave-length region of 250–430 $m\mu$ —the region of characteristic selective absorption by this pigment—by dividing the extinction coefficient for pure gossypol (7) at each wave length by the gossypol content of the glands. The curve constructed with these values (typical example in fig. 1, curve *B*) was identical, within the limits of experimental error, with the experimentally determined curve of the absorption spectrum (typical example in fig. 1, curve *A*) of the chloroform extract of the glands, thus demonstrating the absence of any detectable amounts of a yellow pigment other than gossypol in the glands examined.

Corresponding samples of glands were extracted with chloroform, diethyl ether, methanol, ethanol, and aqueous mixtures of the last two solvents. The absorption spectra of the chloroform solutions of these extracts were identical with one another and with the spectral curve calculated for gossypol (fig. 1, curve *B*) throughout the region from 250 to 430 $m\mu$.

The complete absorption spectra from 240 to 600 $m\mu$ of chloroform extracts of pigment glands (typical example in fig. 1, curve *F*) were almost identical with the composite curves obtained by plotting the values calculated for both gossypol and gossypurpurin in the extracts, thus indicating that these pig-

TABLE 1

COMPARISON OF GOSSYPOL AND GOSSYPURPURIN CONTENT OF SEED KERNELS AND OF CORRESPONDING PIGMENT GLANDS

Nature and variety of products	Gossypol content (%) [*]	Gossypurpurin content (%) [†]	Content of glands in seed kernels (%) [‡]
1. Delfos 651§	1.09	0.055	2.66
2. Pigment glands from (1)	40.9	1.73
3. Cleve wilt	2.39	0.0388	4.81
4. Pigment glands from (3)	49.2	0.612
5. D & P L-45¶	1.14	0.0368	2.92
6. Pigment glands from (5)	39.0	1.08
7. Delfos -451-42-43**	1.11	0.0286	2.37
8. Pigment glands from (7)	43.96	1.08

^{*} Determined by antimony trichloride spectrophotometric method.

[†] Calculated on basis of $E_{1\text{cm}}^{1\%}$ of chloroform extract and $E_{1\text{cm}}^{1\%}$ of pure gossypurpurin, 225.7, at 565–566 $m\mu$.

[‡] Calculated on basis of gossypol content of glands and corresponding seed: ($\%$ gossypol in seed/ $\%$ gossypol in glands) $\times 100$.

§ Pure-bred cottonseed from 1943 crop at Stoneville, Mississippi, stored for 2 years prior to separation of glands.

|| Pure-bred cottonseed from 1943 crop at Clemson, South Carolina, stored for 2 years prior to separation of glands.

¶ Pure-bred cottonseed from 1944 crop at Stoneville, Mississippi, stored for 2½ years prior to separation of glands.

** Pure-bred cottonseed from 1945 crop at Stoneville, Mississippi, stored for 1½ years prior to separation of glands.

ments were the only ones present in detectable amounts in the glands examined.

Aqueous alkali extracted all the color from chloroform extracts of pigment glands. The material recovered by acidification of the alkaline extract gave an antimony trichloride reaction product which was stable for 24 hours and exhibited the absorption spectrum charac-

teristic of the reaction product of pure gossypol.

EXTRAGLANDULAR OIL-SOLUBLE PIGMENTS.—The extinction coefficients for gossypol in each sample of seed kernels investigated were calculated by dividing the value of $E_{1\text{ cm.}}^{\text{gm./l.}}$ for pure gossypol at each wave length throughout the range from 250 to 430 $m\mu$ by the gossypol content of the seed. The curves constructed by plotting the values calculated for gossypol in the seed extracts (typical example in fig. 1, curve D) were significantly lower than the experimentally

determined curves of the absorption spectra of the corresponding extracts of the seed (typical example in fig. 1, curve C).

Anhydrous light petroleum naphtha was used with dried cottonseed flakes to extract the extraglandular, oil-soluble material, free of intraglandular material. The absorption spectrum of the extract (fig. 2, curve B) exhibited a broad maximum at 368–374 $m\mu$ and an inflection at 280 $m\mu$, in contrast to the maxima observed at 362–366, 288–289, and 278–280 $m\mu$ for gossypol in chloroform ex-

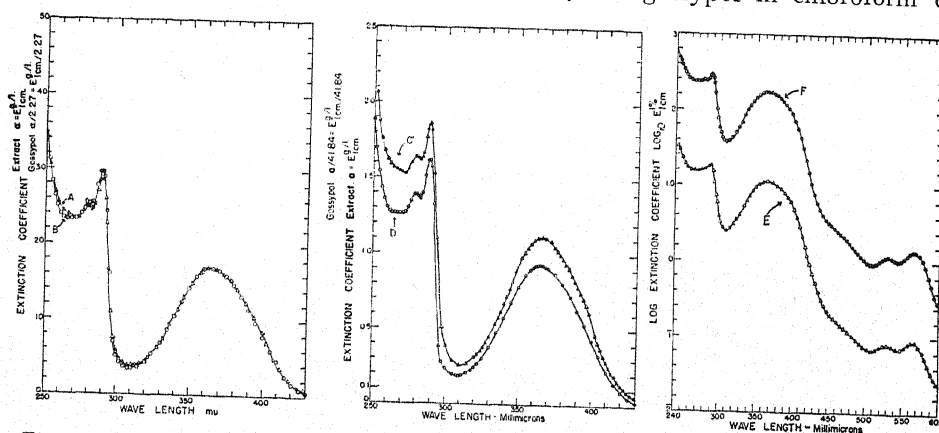


FIG. 1.—Absorption spectra of (A) chloroform extract of cottonseed pigment glands, (B) gossypol in extract of pigment glands, (C) chloroform extract of corresponding kernels, (D) gossypol in extract of kernels, (E) chloroform extract of corresponding kernels, and (F) chloroform extract of pigment glands.

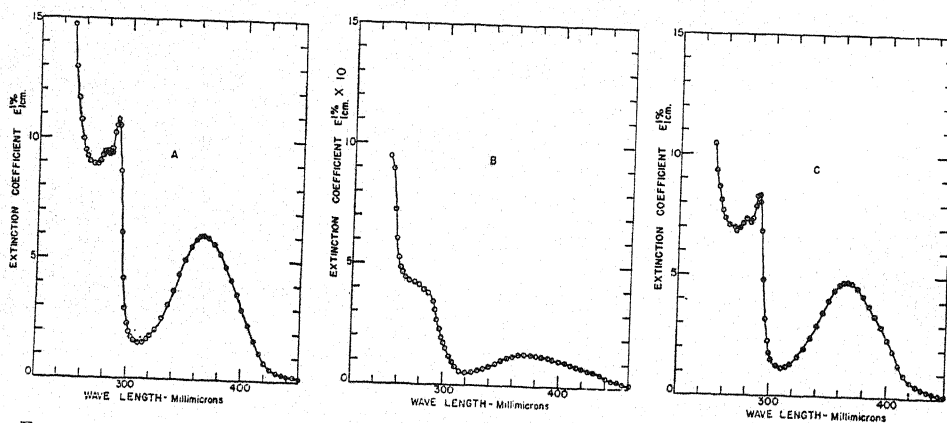


FIG. 2.—Absorption spectra of (A) chloroform extract of cottonseed, (B) petroleum naphtha extract of same seed, (C) chloroform extract of defatted seed.

tracts of the same seed (fig. 2, curve *A*) and of defatted seed (fig. 2, curve *C*).

When special precautions were not taken to reduce the moisture content of seed and solvent, or when extraction was prolonged, or both, some of the glands were ruptured during extraction with petroleum naphtha. Consequently, the absorption spectra of such extracts (fig. 3, curve *A*) indicated the presence of both gossypol and extraglandular pigment. Extraction with aqueous alkali was used to remove gossypol and the decomposition product of gossypurpurin from the petroleum naphtha extract. The absorption spectrum obtained with the residual solution (fig. 3, curve *B*) was the same as that obtained with a petroleum naphtha extract of dry seed (fig. 2, curve *B*).

The amount of gossypol in the original petroleum naphtha extract of moist seed was 0.336% of the weight of extracted oil. The composite curve, based upon the sum of the absorption calculated for gossypol in the extract and the absorption of the residual extract, was slightly lower than that of the original extract. It is not known whether this difference is a result of a shift in the absorption spectrum of the extraglandular pigment in the presence of alkali, or whether it is caused by the removal of a small amount of the extraglandular material during alkaline extraction of gossypol from the extract.

The extraglandular material formed an unstable reaction product with antimony trichloride which exhibited some selective absorption in the visible and near ultraviolet wave-length region which increased in intensity toward the shorter wave lengths. Transitory absorption maxima were usually observed at 420, 450–460, and 490 $m\mu$. The absorption was intensified as the reaction product

aged, the increase being greater toward the regions of shorter wave lengths.

PIGMENTATION OF SOLVENT-EXTRACTED COTTONSEED MEAL.—All the gossypol was extracted from cottonseed of moderate moisture content during contact for a period of 24 hours with commercial-grade chloroform containing

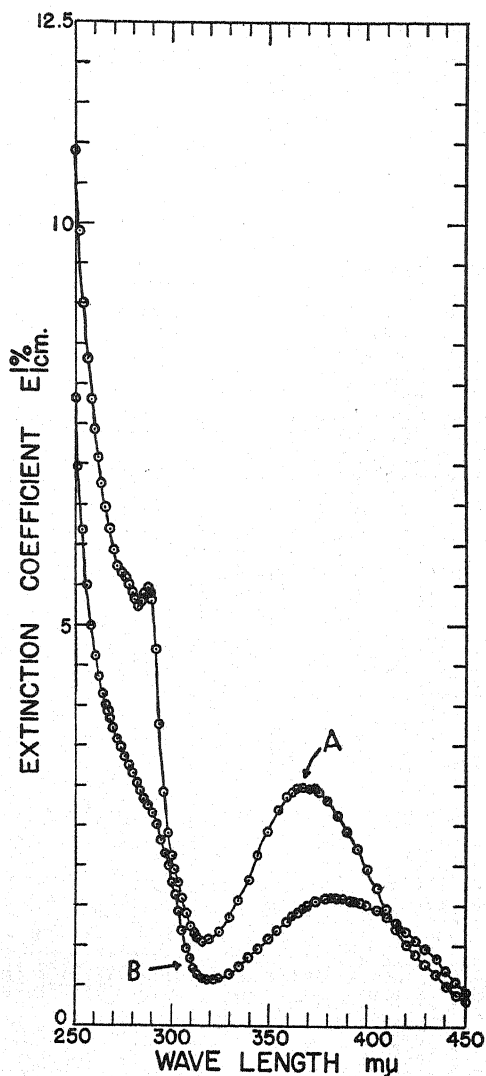


FIG. 3.—Absorption spectra of (*A*) petroleum naphtha extract of moist cottonseed and (*B*) same extract after removal of gossypol by extraction with aqueous alkali.

some moisture (table 2). Dried chloroform extracted only part of the total gossypol present in dried cottonseed during the same period of contact. After preliminary moistening of the seed to rupture the pigment glands, all the gossypol was extracted within a few minutes by chloroform or aqueous ethanol.

Light petroleum naphtha, in which gossypol is very slightly soluble, extracted only a fraction of the total gossypol from cottonseed (table 3), even after preliminary moistening of the seed to rupture the glands. Since dried petroleum naphtha extracted some gossypol from dried flakes, it was apparent that

TABLE 2
EFFECT OF MOISTURE ON EXTRACTION OF GOSSYPOL BY ORGANIC SOLVENTS

SAMPLE	TREATMENT	METHOD OF EXTRACTION			GOSSYPOL EXTRACTED (% OF TOTAL)*
		Preliminary treatment	Solvent	Time of contact (hours)	
Cottonseed (CS-165)	None	None	Chloroform	24	100
Cottonseed (CS-165).....	Dried†	Dried‡	Chloroform	24	40.8
Cottonseed (CS-165).....	None	Ethanol (30%)§	Ethanol (60%)	6	100
Pigment glands (C-78).....	None	None	Chloroform	24	100
Pigment glands (C-78).....	Dried†	Dried‡	Chloroform	24	28.6
Cottonseed (CS-101).....	None	None	Chloroform	2	100
Cottonseed (CS-101).....	Wetted	None	Chloroform	5	100
Cottonseed (CS-926).....	None	None	Chloroform	24	100
Cottonseed (CS-926).....	Wetted	None	Chloroform	20	97
Cottonseed (CS-103).....	None	None	Chloroform	24	100
Cottonseed (CS-103).....	Wetted	None	Chloroform	60	90

* Total gossypol determined by equilibration of untreated seed with chloroform at 38° F. for 24 hours and application of antimony trichloride spectrophotometric method to extract.

† Dried in vacuum desiccator over anhydrous calcium sulfate.

‡ Dried by treatment with calcium chloride.

§ Treated with 30% ethanol (30% ethanol by weight), then 72% ethanol added to yield a final extract of 60% (by weight) ethanol.

|| Flaked or ground seed thoroughly moistened, then dried overnight in vacuum desiccator over anhydrous calcium sulfate.

TABLE 3
EXTRACTION OF GOSSYPOL FROM COTTONSEED BY PETROLEUM NAPHTHA

PREPARATION OF SEED FOR EXTRACTION	TREATMENT OF SOLVENT	CONDITIONS OF EXTRACTION		GOSSYPOL EXTRACTED (% OF TOTAL)*
		Time (hours)	Temperature (° F.)	
Ground meats, wetted, then dried†....	None	20	76	58
Seed hulled and flaked‡.....	None	2	76	32
Flaked meats, dried†.....	Dried§	24	38	12
Seed dried,† hulled, and ground.....	Dried§	24	38	0

* Gossypol determined on basis of antimony trichloride reaction of chloroform extracts of original seed samples and of petroleum naphtha extracts.

† Dried in vacuum desiccator over anhydrous calcium sulfate for 24 hours.

‡ Moisture content of flaked meats, 8.44%.

§ Dried by treatment with calcium chloride.

|| Moisture content of dried seed, 3.85%.

some of the pigment glands had been ruptured during flaking of the seed. Drying of the seed before flaking prevented extraction of gossypol by anhydrous petroleum naphtha.

As another means of comparing the effectiveness of different organic solvents for the extraction of gossypol from cottonseed, the gossypol content of the different solvent-extracted meals was deter-

naphtha (table 4) demonstrates the variety of conditions—namely, moisture content of seed and solvent and duration and temperature of extraction—which determines the amount of gossypol removed from cottonseed by such extraction. Microscopic examination of these meals showed considerable variation in the distribution of pigments remaining after extraction with this solvent. Most

TABLE 4
GOSSYPOL CONTENT OF SOLVENT-EXTRACTED COTTONSEED*

Seed	Solvent	Time of contact (hours)	Gossypol content of meal (%)†
CS-527‡.....	Commercial hexane and tetrachlorethylene§	24	<0.0058
CS-527.....	Diethyl ether	24	0.025
CS-527.....	Petroleum naphtha	6	0.859
CS-527.....	Commercial hexane¶	6	1.91
1610-E-22.....	Commercial hexane and tetrachlorethylene§	24	<0.007
CS-165.....	Commercial hexane and tetrachlorethylene§	24	<0.022
1619-E-6.....	Commercial hexane and tetrachlorethylene§	24	<0.039
1619-E-17.....	Commercial hexane and tetrachlorethylene§	24	0.00**
1630-E-18.....	Diethyl ether	8	<0.032
1630-E-13.....	Diethyl ether	8	<0.032
1630-E-17.....	Diethyl ether	8	<0.052
486-E-77.....	Commercial hexane¶	6	0.80
486-E-27.....	Commercial hexane¶	6	1.90
CS-165.....	Petroleum naphtha	2	1.81

* Commercial grade solvents and seed of moderate moisture content were used.

† Extracted with 30% aqueous ethanol (by weight) followed by 72% aqueous ethanol, giving a final mixture of 60% ethanol; gossypol then determined by antimony trichloride spectrophotometric method.

‡ Moisture reduced to 5%.

§ Pigment glands removed by gland flotation process using mixture of commercial hexane and tetrachlorethylene having a density of 1.378 gm./ml.

|| Some absorption at 520 mμ owing to reaction of interfering substances with antimony trichloride.

¶ Boiling range 146°–158° F.

** Determined by application of antimony trichloride spectrophotometric method to aqueous alkaline extract of chloroform solution of aqueous ethanol extract of meal.

mined. It was thus found that methanol, ethanol, isopropanol, acetone, 1,4-dioxane, and aqueous mixtures of these solvents extracted all the gossypol from cottonseed. Extraction of cottonseed with diethyl ether, or removal of pigment glands from the seed by the flotation method, removed all but traces of gossypol (table 4).

The variation in content of gossypol observed in different samples of cottonseed meal extracted with petroleum

of the meals defatted with petroleum naphtha contained a large number of intact pigment glands. In many cases, however, the extraglandular tissue was colored a distinct yellow, while in others the tissue was almost colorless. It was thus evident that the yellow meals contained much of the pigments which had been discharged from ruptured pigment glands and had not subsequently dissolved in the petroleum naphtha during extraction.

EXTRACTION OF RESIDUAL PIGMENTS FROM DEFATTED MEAL.—As shown by the first two entries in table 5, and as previously established (2), independent extractions of defatted cottonseed meal with chloroform frequently do not yield reproducible results. Since preliminary moistening of the defatted meals, in

TABLE 5

EFFECT OF MOISTURE ON EXTRACTION OF GOSSYPOL FROM COTTONSEED MEAL AFTER PRELIMINARY DEFATTING WITH PETROLEUM NAPHTHA

Meal	Treatment before final extraction	Solvent for extraction	Gossypol (%) [*]
C-101....	None	Chloroform	0.65
C-101....	None	Chloroform	0.35
C-101....	Wetted†	Chloroform	0.17
C-926....	None	Chloroform	1.00
C-926....	None	Diethyl ether	1.09
C-926....	Wetted†	Chloroform	0.29
C-926....	Wetted†	Diethyl ether	0.39
CS-165...	Ethanol (30%)‡	Ethanol (60%)	1.81
CS-165...	None	Chloroform	1.48
CS-165...	Wetted†	Chloroform	0.44
CS-165...	Wetted†	Ethanol (60%)	1.23
CS-56....	Ethanol (30%)‡	Ethanol (60%)	0.85
CS-56....	Wetted†	Chloroform	0.01
CS-527...	Ethanol (30%)‡	Ethanol (60%)	1.92
CS-527...	None	Chloroform	1.38
CS-610...	Ethanol (30%)‡	Ethanol (60%)	0.86
CS-610...	None	Chloroform	0.86

^{*} Gossypol content of extract determined by antimony trichloride spectrophotometric method; values expressed on basis of weight of meal.

† Meal thoroughly moistened, then dried overnight in vacuum desiccator over anhydrous calcium sulfate.

‡ Treated with 30% aqueous ethanol (30% ethanol by weight), then 72% aqueous ethanol added to yield final extract of 60% ethanol.

order to rupture the pigment glands, was found to reduce the amount of gossypol extractable with chloroform or diethyl ether (table 5), it was apparent that the resistance of the gland walls was not solely responsible for the difficulty encountered in removing the residual pigments from defatted meals by extraction

with these solvents. After preliminary rupture of the gland walls by the use of water or aqueous ethanol mixtures of high water content, the gossypol was completely and rapidly extractable with ethanol. The residual meals were almost completely colorless except those which had contained relatively large amounts of gossypurpurin. Treatment with methanol, isopropanol, acetone, or 1,4-dioxane was also found to remove all the gossypol from defatted meal. Only the two last-mentioned solvents were effective in removing all the residual gossypurpurin or its yellow decomposition product from defatted cottonseed.

Discussion

Application of the recently developed flotation process (4, 23) for separation of intact pigment glands from other parts of the seed has provided material for the determination of the content of pigments of the glands separated from four samples of different varieties of *Gossypium hirsutum*. Gossypol was found to constitute from 39.0 to 49.2%, and gossypurpurin from 0.612 to 1.73%, of the weight of the separated glands. The demonstration that all the gossypol and gossypurpurin of the kernel is concentrated in the pigment glands has made possible accurate calculation of the average weight of glands in each sample of seed investigated. The average content of glands was found to vary from 2.37 to 2.92% of the weight of the seed containing smaller amounts of gossypol and was 4.81% of the weight of the seed containing the largest amount. Thus, a general correlation has been established between the content of gossypol and of glands in the seed. Since these seed lots were grown under different environmental conditions and were stored for different periods of time, it would appear that the amount of

gossypol in the glands is relatively constant; the gossypol content of the seed is, therefore, largely determined by the content of glands. The variation in the gossypurpurin content of glands separated from different samples of cottonseed is consistent with PODOL'SKAYA'S (16, 17) observation that this pigment can be detected only in maturing and stored seed and that it varies greatly during these periods.

SMIRNOVA (21) has reported that genetic factors are more important than environmental factors in determining both the average number of glands and the content of gossypol in the cottonseed kernel. Seed from different varieties of *G. herbaceum* grown under different conditions contained only a small number of glands and very little gossypol. Seed of different varieties of *G. hirsutum* were intermediate in the average number of glands and in gossypol content, while seed from different varieties of *G. barbadense* contained the largest number of glands and the largest amount of gossypol.

The seed lots examined during the present investigation were all of the same species, and the selection of varieties was too limited to permit differentiating between environmental and genetic factors. However, because of the wide variation in size of pigment glands in different parts of the kernel and in different samples of seed, more accurate correlations can be expected between the relative weights of glands and gossypol than between the number of glands and the weight of gossypol in the kernel, as proposed by SMIRNOVA.

Gossypol and gossypurpurin are the only pigments which could be detected in the extracts of the limited number of samples of pigment glands examined. The absence of these pigments in dry

petroleum naphtha extracts of dry cottonseed indicated that they are segregated in the glands. Incontrovertible evidence for the presence of other pigments in the glands can be obtained only by their actual detection. In the absence of this direct evidence, however, deductions concerning the localization of other pigments can be based on indirect evidence concerning the relative difficulty with which the pigment is extracted from cottonseed. Gossyfulvin (3, 7) was not detected in any of the extracts of pigment glands examined but it does not appear to be of very frequent occurrence in cottonseed. Since prolonged contact with chloroform or ether has been reported (2) to be necessary for extraction of gossyfulvin from seed in which it occurs, it can be inferred that this pigment occurs in the glands rather than in the surrounding tissue. The observation that aqueous alkali extracts all the color from chloroform extracts of separated glands, coupled with the observed stability of the antimony trichloride reaction product of such extracts, provides reliable evidence that none of the yellow, oil-soluble, non-acidic pigment detected in cottonseed extracts occurs in the glands.

The absorption spectra of chloroform extracts, and of chloroform solutions of diethyl ether and aqueous ethanol extracts, of several samples of pigment glands separated from different lots of cottonseed were found to be identical with the absorption spectra calculated for gossypol in the extracts (fig. 1, curves A, B). Thus it would appear that the structure of gossypol is not altered during its extraction from the glands and subsequent purification. It can further be deduced that gossypol is not chemically combined with any of the other components of the glands but occurs as a colloidal, gelatinous suspension in the

glands. Because of the extreme instability of gossypurpurin in solvents other than chloroform, the absorption spectrum of the isolated pigment could be compared only with that of chloroform extracts of pigment glands.

The yellow extraglandular material is obtained free of intraglandular pigments by treating dry seed with dry petroleum naphtha; this procedure avoids rupture of the glands. Moreover, since the extraglandular pigment is nonacidic, it is not extracted from its solutions in organic solvents by treatment with aqueous alkali, whereas both gossypol and gossypurpurin, and the yellow decomposition product of gossypurpurin, are quantitatively extracted from such solutions. The absorption spectrum and chemical properties of this newly detected pigment differentiate it from gossypol, gossypurpurin, the yellow decomposition product of gossypurpurin, gossyfulvin, and the oil-soluble carotenoid pigment reported by PODOL'SKAYA (15, 18). Since this pigment is apparently stable in solution in the oil in contact with the reactive constituents of the seed tissue, it can be inferred that it is a relatively inert, non-polar compound. Investigations (8) of its behavior in cottonseed oil extracted with petroleum naphtha indicate that it is essentially unaffected during alkali refining and subsequent bleaching of the oil. The carotenoid pigment reported by PODOL'SKAYA could not be detected in any cottonseed extract examined. However, since this pigment is readily extracted from cottonseed by petroleum naphtha, it can be inferred that in the seed in which it occurs it is found in solution in the oil surrounding the pigment glands.

Solvent-extracted cottonseed meals have been shown to fall into several categories with respect to the distribution and total content of pigments. Treatment of flaked seed with water-miscible

alcohols, ketones, and ethers of low molecular weight, or with aqueous mixtures of any one of these solvents, ruptures the pigment glands. The pigment content of the residual meal is therefore determined entirely by the solubility of the pigments in the solvents or solvent mixtures. Acetone and dioxane extract all the pigments and yield almost colorless meals. Because of the very slight solubility of gossypurpurin and its yellow decomposition product in the alcohols, these solvents remove all the pigments with the oil only when the original seed contains relatively small amounts of gossypurpurin.

If moisture is rigorously excluded, the meals remaining after extraction of the oil with organic solvents, other than water-miscible alcohols, ketones, and ethers of low molecular weight, contain none of the oil-soluble extraglandular pigments, but most of the intraglandular pigments remain in intact pigment glands with the meal. With the use of moist solvents in which the pigments are soluble, such as commercial-grade chloroform and diethyl ether, the pigment glands are slowly ruptured and most of the pigments are removed from the meal during prolonged extraction. Preliminary wetting of the seed in order to rupture the pigment glands renders the pigments immediately extractable with the aforementioned solvents.

The extent to which the pigments are extracted from the seed by solvents in which they are not very soluble, such as the petroleum naphthas, will depend upon the conditions of extraction. When the pigment glands are ruptured by moisture in the seed before extraction of the oil, a relatively large proportion of the pigments will be dissolved in the oil and solvent mixture to which they are exposed during the initial stages of the extraction. Most of the pigments which are

discharged from pigment glands (ruptured during prolonged extraction and removal of most of the oil) are not removed by the extracting solvent but remain adsorbed on the meal. Consequently, meals obtained by extracting the oil with petroleum naphtha in the presence of moderate amounts of moisture will contain part of the pigments in the intact pigment glands and part will be adsorbed on the extraglandular tissue.

The adsorbed pigments can be removed from defatted cottonseed meal only by treatment with polar organic solvents. Treatment of such meals with alcohols or aqueous mixtures thereof removes all the adsorbed gossypol as well as that contained in the intact glands, but these solvents remove only small amounts of the yellow decomposition product of gossypurpurin. Meals which are essentially free of color were obtained by supplementary extraction of defatted meals with acetone or 1,4-dioxane, even when the original seed contained relatively large amounts of gossypurpurin.

Summary

1. All the gossypol and gossypurpurin of the cottonseed kernel are segregated in the pigment glands, and they constitute the only detectable pigments in the glands of four varieties of seed of *Gossypium hirsutum* which were examined.

2. The gossypol content of the glands is relatively constant, this pigment constituting up to approximately 50% of the

weight of the glands, whereas the gossypurpurin content is relatively low and variable.

3. On the basis of the direct correlation observed between the amounts of glands and of gossypol in the kernels, it is suggested that the gland content is the principal factor which determines the gossypol content of the cottonseed kernel.

4. A yellow pigment has been detected in solution in the oil of the extraglandular tissue of the kernel. This pigment has been obtained free of gossypol, gossypurpurin, and the yellow decomposition product of gossypurpurin. Partial characterization of the extraglandular pigment has shown it to be relatively stable and to differ from every pigment previously detected in cottonseed.

5. On the basis of the distribution and properties of the pigments of cottonseed, methods for their extraction and estimation have been developed.

The authors wish to acknowledge the generosity of J. WINSTON NEELY and W. R. PADEN in providing the samples of pure-bred seed with which these investigations were carried out. They also wish to thank H. L. E. VIX, J. SPADARO, E. F. POLLARD, and E. A. GASTROCK for the preparation of some of the samples of separated pigment glands, and MILDRED MURRAY for some of the spectrophotometric data.

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INFLUENCE OF PHOTOPERIOD ON MICROSPOROGENESIS IN COSMOS SULPHUREUS CAV. VAR. KLONDIKE¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 590

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Introduction

Degenerative cytological changes induced by environmental effects on microsporogenesis and upon the development of pollen grains have been reported by a number of investigators (7, 8, 10, 12, 13, 14, 15, 16).

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Photoperiodic effects on microsporogenesis in Biloxi soybean were investigated by NIELSEN (11). Plants given 16- or 21-hour postinductive photoperiods following fewer than six photoinductive cycles failed to show development beyond the early prophase stages of meiosis. Those receiving six to ten photoinductive cycles showed degenerative changes, such as enlarged sporocytes with highly

vacuolate cytoplasm, heavily staining sporogenous tissue, failure of telophasic reconstruction, degenerate microspores, and abnormal pollen.

In the classical investigations on photoperiod by GARNER and ALLARD, *Cosmos* was classed as a short-day plant (4). When sufficiently photoinduced, flowering begins at the apex and extends progressively downward along the stem (6). However, inhibition in the development of flower buds of *C. bipinnatus* was obtained by the use of long photoperiods after sufficient induction by short-day cycles (5). This retarding effect of subsequent treatment with long photoperiods was established later for *C. sulphureus* (2). Photoperiodic response is of a strictly localized nature in this species, since buds develop and flower only on the photoinduced portion or one node beyond (6).

The quantitative effect of the length of photoperiod manifested itself in data collected by AUSTIN (1). In plants treated with 8-hour light periods, buds appeared 11 days earlier than in plants on 12-hour photoperiod; after flower bud initiation there was no differential effect on floral development. Ten $7\frac{1}{2}$ -hour photoperiods are sufficient to bring about the production of flowers and seeds in *Cosmos* (2). This reproductive phase is accompanied by a reduction in catalase activity (9) and by a marked accumulation of carbohydrate and protein in the stem tip (2). When fewer inductive photoperiods are followed by long-day cycles, the floral primordia tend to develop into "vegetative flowers" or interphases between normal flowers and vegetative shoots. If the induction is sufficiently limited, reversion to vegetative growth may occur. These anatomical changes toward foliaceous floral structures have been described by BIDULPH (2).

Although flower buds appear on *Cosmos* exposed to twelve short light periods, STRUCKMEYER's work (17) indicated that eighteen short photoperiods are required to prevent the development of interphases or "vegetative flowers." Anatomical analysis of the stem showed that under photoinductive treatment cambial activity was decreased; when the plant was returned to an environment conducive to vegetative growth, meristematic activity was again resumed and was sometimes accompanied by the aborting of floral primordia.

Recent investigations (3) on *Cosmos* indicate that the "stimulus to flowering" produced during photoperiodic induction continues to pass from the leaves after they are returned to long photoperiods.

These anatomical and morphological changes in *Cosmos*, as responses to photoperiodic induction and postinduction treatment, suggest an attendant cytological upset with which this present study deals.

Material and methods

Seeds of *Cosmos sulphureus* Cav. var. Klondike (a short-day strain) were planted in flats on greenhouse benches on July 31, 1946. Immediately after germination the seedlings were placed on long photoperiods (8:00 A.M.—3:00 A.M.) obtained by supplementing the natural daylength with "Daylight" fluorescent lamps automatically controlled by clocks. When 2 weeks old, the seedlings were planted singly in 2-inch pots. On August 24 they were selected for uniformity of size and vigor and were transplanted in pairs into 4-inch pots.

On September 21, when all plants had at least five pairs of true leaves, twenty-three lots of nine pots each (eighteen plants) were subjected to differential light treatments (table 1). Six groups of

three lots each were placed on 8-hour photoperiods for 6, 7, 8, 10, 12, or 14 days, respectively. The three lots in each group received subsequent photoperiods of 14, 19, or 24 hours. Five lots of controls were employed. One lot was placed on natural daylength, another on 8 hours of light and 16 hours of darkness; both of these flowered profusely. The other controls on 14-, 19-, and 24-hour photoperiods did not flower. The photoinductive cycles of 8 hours of light and 16 hours of darkness were obtained by

covering bench frames, built over the plants, with heavy black cloth at 4:00 P.M. and removing the cover at 8:00 A.M. the next morning. All plants were kept in two rooms of the same wing of the greenhouse in order to obtain environmental conditions that were as nearly identical as possible until the variable of light was introduced.

Floral buds were fixed in SAX's modification of Navashin's solution, washed in tap water, and run up in an ethyl-tertiary butyl alcohol dehydrating series

TABLE 1
SUMMARY OF TREATMENT AND HARVEST

Group no.	Placed on short photoperiod	Placed on long photoperiod	Total no. of photoinductive cycles	Postinduction treatment	Total no. of buds developed	First harvest	Last harvest
I.....	9/21/46	9/27/46	6	{ A. 14 hr. C. 19 hr. D. 24 hr.	2 1 1	11/18/46 12/14/46 1/14/47
II.....	9/21/46	9/28/46	7	{ A. 14 hr. C. 19 hr. D. 24 hr.	14 8 8	11/ 8/46 11/ 7/46 11/ 5/46	1/22/47 1/ 2/47 1/ 2/47
III.....	9/21/46	9/29/46	8	{ A. 14 hr. C. 19 hr. D. 24 hr.	22 19 15	11/ 5/46 11/ 7/46 10/30/46	1/12/47 1/12/47 1/ 2/47
IV.....	9/21/46	10/ 1/46	10	{ A. 14 hr. C. 19 hr. D. 24 hr.	25 28 17	11/ 4/46 10/31/46 11/ 4/46	1/ 2/47 1/12/47 1/12/47
V.....	9/21/46	10/ 3/46	12	{ A. 14 hr. C. 19 hr. D. 24 hr.	51 49 39	10/26/46 10/26/46 10/22/46	1/12/47 1/12/47 1/12/47
VI.....	9/21/46	10/ 5/46	14	{ A. 14 hr. C. 19 hr. D. 24 hr.	85 88 83	10/15/46 10/26/46 10/16/46	1/12/47 1/12/47 1/12/47

CONTROLS

Group no.	Placed on short photoperiod	Placed on long photoperiod	Photoperiod	First harvest	Last harvest
VII.....	9/21/46	Natural	10/14/46	11/22/46
VIII.....	9/21/46	8 hr.	10/10/46	11/11/46
IX.....	9/21/46	14 hr.
XI.....	9/21/46	24 hr.
XII.....	9/21/46	19 hr.

into paraffin. Sections cut at 10μ were stained with Heidenhain's iron-alum haemotoxylin.

Observations

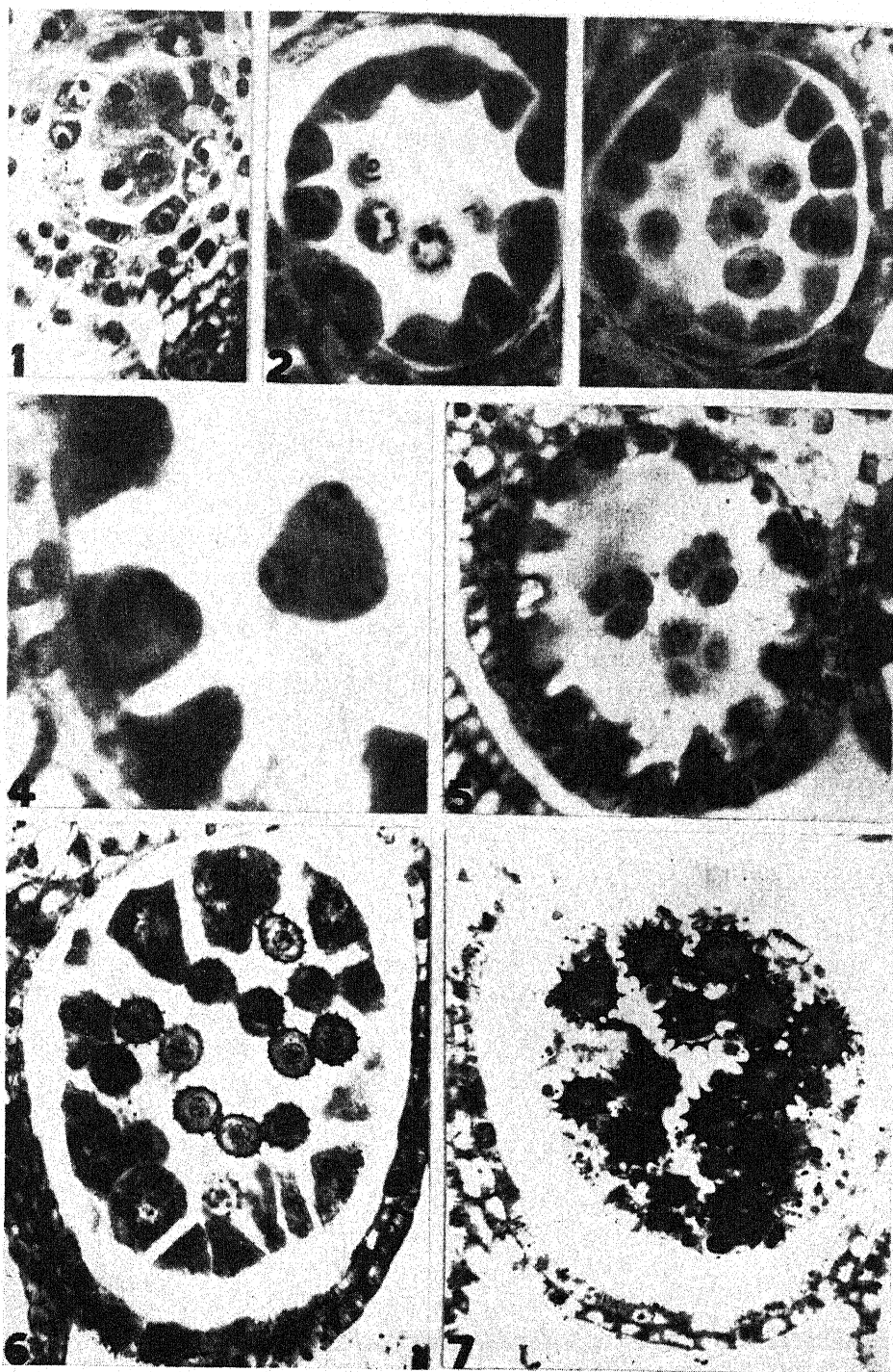
The normal flower head of *Cosmos* develops two rows of involucre bracts, consisting usually of eight in each row. The inner membranous bracts, surrounded by other foliaceous ones, bear the usual eight-ray flowers in their axils. The disk flowers are produced in the axils of bractlets on the determinate head. The young floral heads are completely inclosed by the inner membranous set of involucre bracts, while the outer bracts are spreading ones (fig. 31). In the development of the flower bud, the floral tube is usually differentiated first but is closely followed by the stamen primordia in which the microsporocytes soon differentiate. The sporocyte cells are larger and have slightly denser cytoplasm and more prominent nuclei than the surrounding somatic cells. Continued division of the sporogenous cells, which soon become angular in contour, is accompanied by the differentiation of the sporangial wall and tapetum (fig. 1).

As growth proceeds, the reticulum of the metabolic nucleus gives way to the attenuated chromonemata of the leptotene, scattered throughout the nucleus. The pairing of the slightly contracted threads and an increased volume of the nucleus characterize the xygotene stage. Continued contraction of the paired chromosomes (pachytene) and further enlargement of the nucleus proceed as the cytoplasm of the microsporocytes rounds up. The rounding-up is completed in the diplotene when the chromosomes have contracted still further and are evenly distributed near the nuclear membrane (fig. 2). Marked shortening of the chromosomes (diakinesis) is immediate-

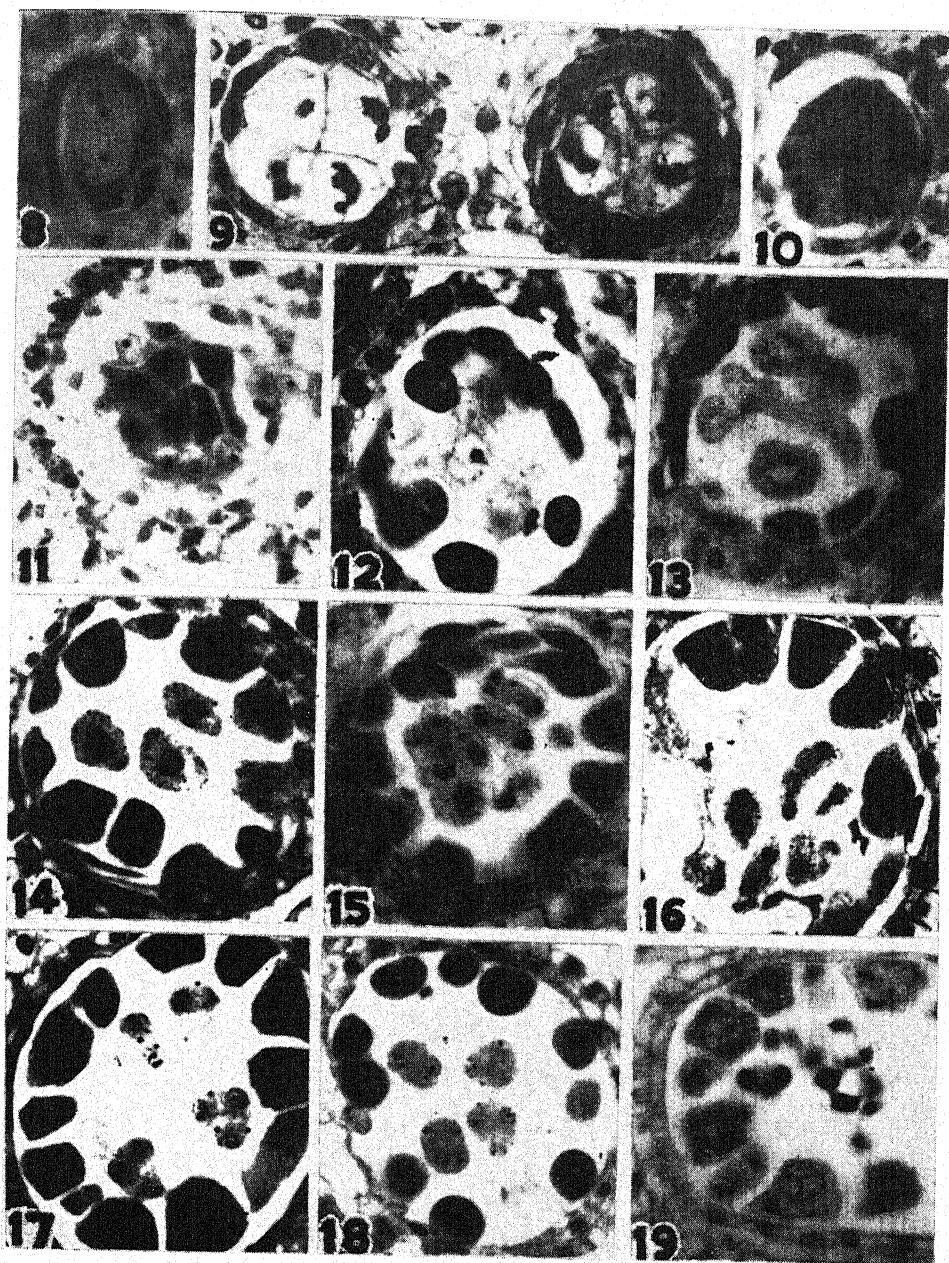
ly followed by the alignment of them at the equatorial plate (metaphase I, fig. 3). The disjoining of the chromosomes (anaphase I) results in the formation of dyad nuclei. Concurrent with the developing prophase stages are the division and the enlargement of tapetal cells.

The second meiotic division follows immediately, giving rise to the four-nucleate stage (fig. 4). Cytokinesis produces a quartet of spores (fig. 5), and, as these round out, the walls of the microsporocytes disintegrate. The tapetal cells disintegrate and the residue from them diffuses into the locular cavity, producing a translucent, granular matrix in which occurs the transition from thin, smooth-walled microspore to thick, spiny-walled pollen grain (figs. 6, 7).

The individuals most responsive to the photoinductive cycles in groups IV, V, and VI produced morphologically normal terminal flowers. The less responsive in these groups, as well as the most sensitive in groups II and III, yielded floral heads of smaller proportions with longer than normal foliaceous bracts (figs. 32, 33, 34). The floral buds which were initiated later developed at varying rates in each group. Some of the primordia remained in an undifferentiated condition, while others early differentiated the floral tube and stamens containing sporogenous cells and tapetum. In groups II-VI the first apparent degenerative changes seemed to occur in the tapetum. These changes began as early as the metabolic nuclear stage of the microsporocytes and were evident as abnormally narrow cells (figs. 8, 9). As degeneration of the tapetal cells proceeded, their cytoplasm stained black, and the cells sometimes became oval in shape (fig. 12). More frequently, however, they remained narrow (figs. 8, 22, 23) or became angular (figs. 13, 14) in



FIGS. 1-7.—From control plants of group VIII. Fig. 1, young microsporocytes, normal tapetum, and sporangial wall. Fig. 2, diplotene. Fig. 3, metaphase. Fig. 4, four-nucleate stage. Fig. 5, quartet of microspores. Fig. 6, microspores. Fig. 7, pollen grains.



FIGS. 8-19.—Fig. 8, microsporocytes surrounded by degenerating tapetal cells, lot IV-D. Fig. 9, microsporocytes with little or no cytoplasm, surrounded by darkening tapetum, lot IV-A. Fig. 10, sporogenous tissue darkening, tapetum narrow and degenerating, lot II-D. Fig. 11, one locule with degenerating microsporocytes and outer sporangial wall containing heavily staining globular bodies, lot III-D. Fig. 12, locule of anther with globular tapetal cells and disintegrating sporocytes, lot IV-C. Fig. 13, locule containing narrow and angular tapetal cells inclosing normal microsporocyte and large denuded plasmodial mass, lot IV-A. Fig. 14, locule containing angular degenerating tapetal cells with heavy black granules floating in cytoplasm, lot IV-D. Fig. 15, degenerating tapetum and lagging chromosomes in meiosis I, lot II-A. Fig. 16, degenerating tapetum and disintegrating dyads with extra-nuclear chromosomes, lot II-A. Fig. 17, degenerating tapetum and tetrads with extra-nuclear chromosomes, lot IV-D. Fig. 18, rounded, disintegrating tapetal cells and tetrads with extra-nuclear chromosomes, lot IV-A. Fig. 19, incipient stages of tapetal degeneration and advanced degeneration of dyads and tetrads, lot II-C.

outline. Less often observed were the darkly stained microsporocytes and vacuolated cells of the tapetum as shown in figure 10.

Degenerating cytoplasm in microsporocytes was of common occurrence. In a few cases heavily staining globular bodies in the outer sporangial wall accompanied these deteriorating microsporocytes (fig. 11). Coincident with these responses of the tapetum were two degenerative reactions of the sporogenous cells. In the extremely suppressed floral buds of groups I-VI the prophase seemed not to be initiated; the cytoplasm of these microsporocytes became highly vacuolate with the nucleus markedly contracted. Such cells appeared to develop no further (figs. 9, 29). On the other hand, in less suppressed buds of groups II-VI the cytoplasm of the sporogenous cells sometimes appeared normal and prophase might continue regularly; frequently some of the sister sporocytes developed into large plasmodial masses which were two to four times the average size of normal microsporocytes (fig. 13).

At the time of late diakinesis and metaphase I, a condition frequently observed in the microsporocytes in groups II-VI was the occurrence of darkly staining cytoplasm with scattered, heavily stained, black granules (fig. 14). A less frequent abnormality was the lagging of chromosomes in anaphase I (fig. 15). These chromosomes could still be seen after the second meiotic division was completed (fig. 17). This occurrence was also noted in association with rounded tapetal cells (fig. 18). Where flower suppression was greater, degeneration often took place at the two-nucleate stages and chromosomes could be seen floating in the dark-stained cytoplasm near the partially reconstituted nuclei. Shapeless

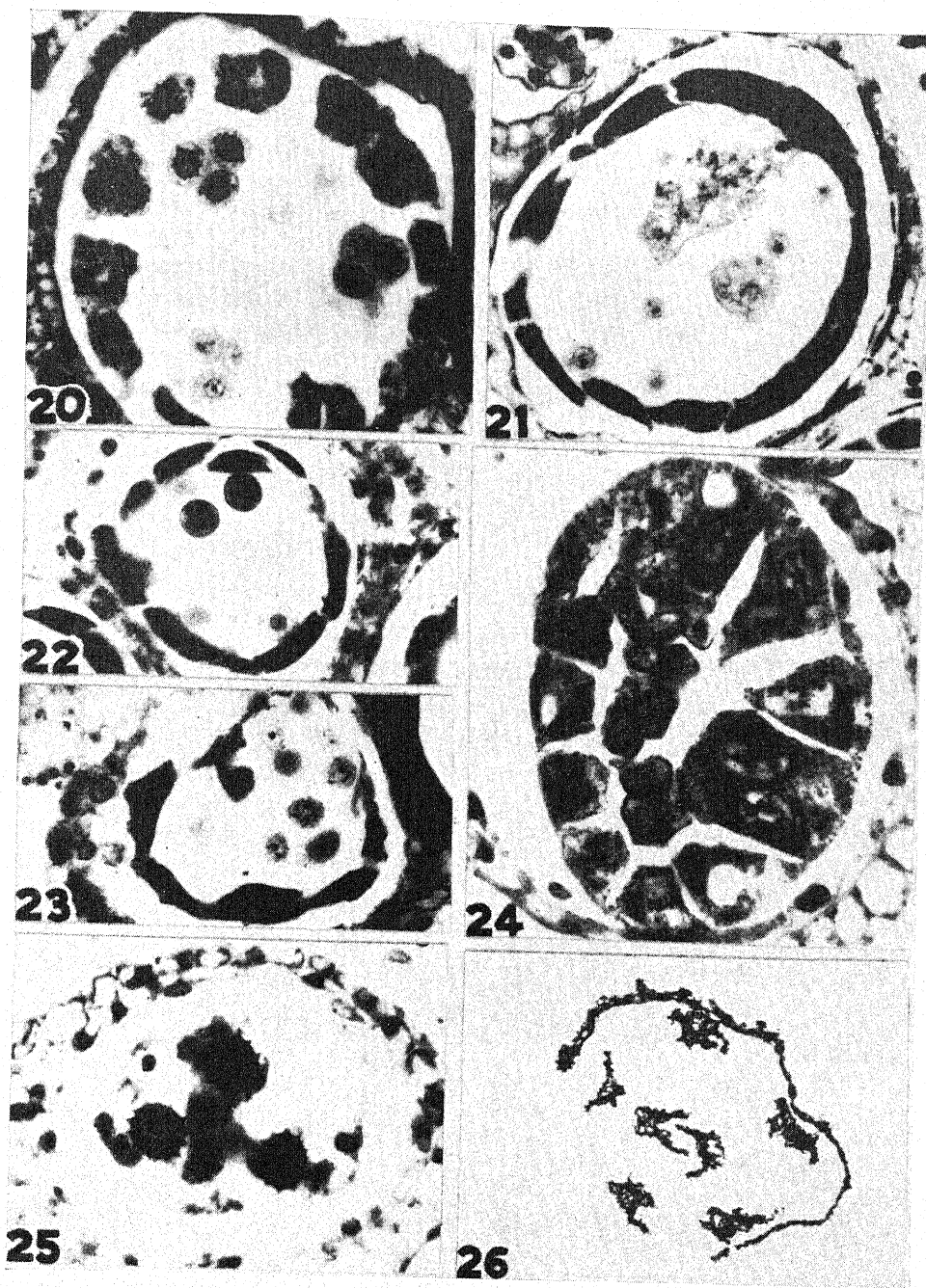
masses of both heavily stained and highly vacuolate protoplasm also occurred (fig. 16).

Although degeneration of sporogenous tissue at any stage of development was usually preceded or accompanied by marked deterioration of tapetal cells, occasionally a severe upset in meiosis occurred, although tapetal cells showed only the incipient stages of cytoplasmic degeneration (fig. 19).

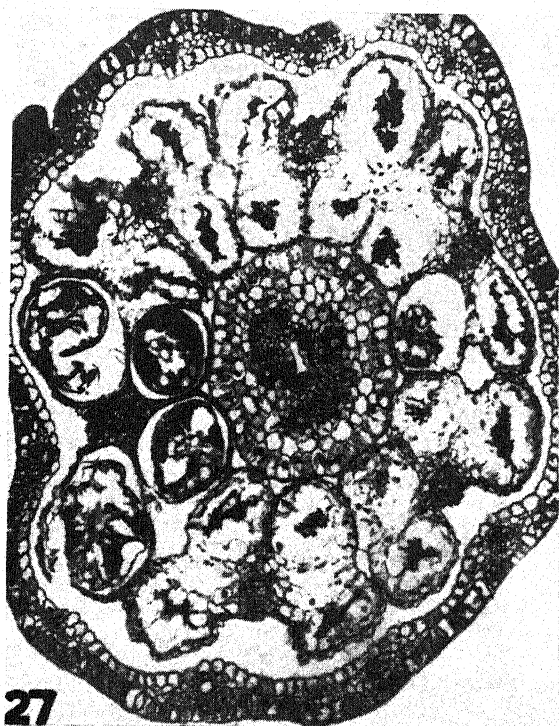
In the plants in groups II-VI in which buds developed to a fuller extent than did those just mentioned, and lagging of chromosomes was not apparent, cytoplasmic changes first appeared immediately exterior to the intensely stained nucleus (fig. 20). Position and procedure of degeneration were similar in both tetrads and microspores (fig. 22). Of less frequent incidence, apparently normal microspores occurred in conjunction with irregular masses of granular cytoplasm that showed evidence of almost complete nuclear impairment (fig. 21). When degeneration of tapetal cells was as marked as is shown in figures 21, 22, and 23, many microspores did not develop further. With less deterioration in the tapetum, the microspore wall began to thicken and the spores became angular in appearance (fig. 24).

Groups II-VI produced a few flowers which yielded some morphologically normal pollen. However, in the locules containing an abnormally dark-staining matrix small, shrunken, black-stained bodies (deteriorated microspores) appeared along with apparently normal pollen grains (fig. 25).

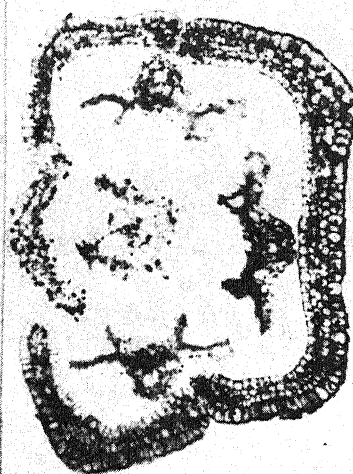
Groups I-VI also showed more pronounced degeneration than just described, in which the locules were filled with an indistinguishable, deteriorated mass of sporogenous tissue. The terminal flower bud from lot II-C, having seven



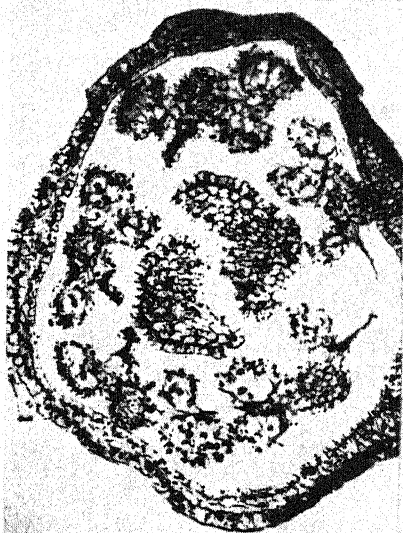
FIGS. 20-26.—Fig. 20, locule with deteriorating tapetum surrounding quartets of spores which show cytoplasmic degeneration immediately exterior to nuclear membrane, lot V-D. Fig. 21, locule with narrow, blackened tapetal cells surrounding a few normal microspores and two plasmodial masses with evidences of deteriorated nuclear material, lot III-A. Fig. 22, locule of anther with narrow, degenerating tapetal cells and degenerating microspores, lot II-C. Fig. 23, locule containing darkening sporangial wall, degenerating tapetum, and deteriorating microspores, lot IV-A. Fig. 24, locule with abnormally darkened tapetal cytoplasm filled with large granules and disintegrating angular microspores, lot II-D. Fig. 25, locule containing several morphologically normal pollen grains, many shrunken grains, and heavily staining tapetal matrix, III-A. Fig. 26, flower from lot II-A with shriveled anthers and floral tube.



27



28



29



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FIGS. 27-30.—Fig. 27, one flower from floral head in fig. 33; stages of tapetal and sporogenous degeneration, lot II-C. Fig. 28, flower from floral head in fig. 34 with three stamens of shriveled anthers and floral tube, lot III-D. Figs. 29, 30, flowers from two first-node (below terminal) buds of same plant, lot V-D; fig. 29, flower having locules with vacuolated sporogenous tissue; fig. 30, flower from floral head in fig. 32 with partially collapsed locules containing abnormal pollen.

photoinductive cycles followed by a 19-hour photoperiod, illustrates this condition. This head with longer than normal foliaceous bracts and inhibited membranous ones (fig. 33) produced disk flowers of the type pictured in figure 27. Notable is the fact that the locules shown are in different stages of development and deterioration. The most advanced type of deterioration shows mere rudiments of shriveled anther walls, filaments, and floral tubes (fig. 26). Not uncommon were the abnormal numbers of three and four stamens instead of five per flower. A terminal bud (fig. 34) in lot III-D, having eight photoinductive cycles followed by continuous light, produced a number of three-stamen flowers (fig. 28). For the most part in these entirely suppressed heads all the flowers were in the same stages of degeneration. However, in instances of less retardation, differences in stage of development of flowers within one head led to a range from abnormalities in early microsporogenous tissue to small, collapsed pollen grains.

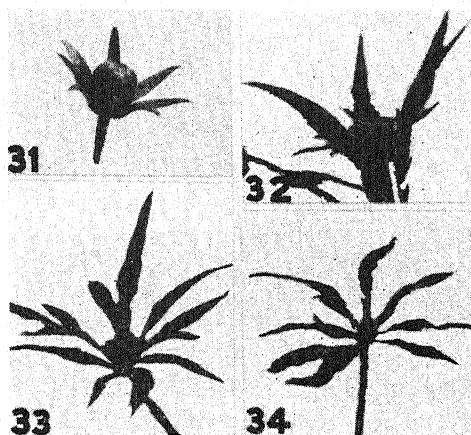
The gross floral response of a first-node (below the terminal) flower to twelve photoinductive treatments, followed by continuous light, is shown in figure 32. The foliaceous bracts were 10–15 mm. in length; the membranous ones were completely inhibited, resulting in a head of unprotected, widely separated flowers. Cytological examination revealed that this bud, the larger of two first-node buds, had produced abnormal pollen (fig. 30), while the smaller one had been inhibited in its development before the initiation of the prophase (fig. 29). These early sporogenous stages showed cells from which the cytoplasm had completely disappeared.

In these experiments a few buds were produced in the six-cycle material in con-

trast to the results obtained by other workers (2, 3). Two of these buds showed collapsed sporangial walls containing degenerate pollen, and the others were not developed beyond the early differentiation of the floral tube.

Discussion

The effects of an insufficient number of short-photoperiod cycles in *C. sulphureus* Cav. var. Klondike are similar



FIGS. 31–34.—Fig. 31, terminal bud from control group VIII. Fig. 32, first-node bud from lot V-D. Fig. 33, terminal bud with long foliaceous outer involucral bracts and inhibited inner one from lot II-C. Fig. 34, terminal bud with abnormally long foliaceous outer bracts and partially inhibited inner ones from lot III-D.

in many respects to those described by NIELSEN (11) in Biloxi soybeans having five to ten photoinductive cycles followed by a long photoperiod. His results showed that sporogenous tissue failed to develop further than the early prophase of meiosis in plants given five or fewer short photoperiods. Under this condition the sporocytes enlarged, became vacuolated, eventually lost their nuclei, and disintegrated. Corresponding consequences were found in carbohydrate-deficient tomatoes, as reported by HOWLETT (8). Severe carbohydrate deficiency

produced a retarding effect on the development of the flower with resultant collapse of cell walls and the degeneration of sporogenous cells before initiation of the prophase. The degree of degeneration was correlated with the severity of the deficiency.

Soybean plants given six to ten short photoperiods frequently showed degeneration at later stages in meiosis than did the five-cycle groups. Buds from these plants showed deterioration after the first division, such as failure of reconstitution of the four chromosome groups following meiosis II, or frequent degeneration of microspores after formation in the tetrad. Some plants receiving eight or more inductive cycles produced a small percentage of morphologically normal microspores. It was concluded that in soybean the number of short photoperiods influences the degree of degeneration and the stage of development to which microsporocytes may progress. The long postinductive photoperiod appeared to effect further the suppression of flowers and the degenerative changes observed (11).

In the markedly suppressed buds of *Cosmos*, no differentiation of flower parts and sporogenous tissue occurs, although grossly a rounded protuberance can be recognized. A somewhat less retarded floral structure shows differentiation of the floral tube, anthers with well-developed sporangial wall, threadlike black tapetum, and sporogenous cells with little or no cytoplasm (fig. 29). This condition in sporogenous tissue concurs with data on tomato (8) and soybean (11) in which development of the sporogenous cells fails to reach the prophase stages of meiosis.

In cases in which the prophases were initiated in the sporogenous tissue in tomato, lagging chromosomes in meiosis

II were reported. Likewise in *Cosmos*, similar lagging was noted in both meiotic divisions in groups II, IV, and V and was always accompanied by a heavily stained tapetum. Failure to proceed beyond meiosis I was observed in many cases (fig. 16). On the other hand, if reconstruction of tetrad nuclei did occur, chromosomes were often found in the cytoplasm outside of the nuclear membrane (figs. 17, 18).

Cells with fully reconstituted nuclei may develop further, but degeneration of microspores often occurred directly following cytokinesis, as in soybean, or later before the microspore wall thickened. Growth of the microspore wall and production of spiny protuberances of the pollen-grain wall are apparently subordinate to the presence of at least some matrix formed from the contents of the tapetal cells. Unless floral development is suppressed sufficiently to prevent initiation of the prophases in all flowers, morphologically normal pollen may be produced in a large percentage of flowers along with numerous degenerative developmental stages.

Since the flowers of *Cosmos* are produced in a progressive fashion from apex downward along the stem (6), comparisons of all terminals, or all first-node flowers, or all second node flowers below the terminal, were made of the experimental material. However, in so far as all the plants are not equally sensitive (2) and the stimulus to flowering is inactivated by young expanding leaves present during the induction period (3), a wide range of variation was encountered microscopically. This was true, not only within an experimental lot, but within buds of the same node on one plant (figs. 29, 30) as well as within the same flower (fig. 27). This considerable overlapping makes it impossible to draw lines

of demarcation cytologically, between the differentially treated lots, even though a quantitative record of buds of the experimental lots expresses a retardation in total reproductive activity in correspondence with reduced photoinduction. An inhibitory effect of the long postinductive cycles on total flower bud production is suggested in the column of bud numbers in table 1. These results are in accord with those obtained in *Cosmos* by BIDDULPH (2) and in soybean by NIELSEN (11).

When *C. sulphureus* plants are treated with a sufficient number of short-photo-period cycles, a series of chemical reactions appears to be initiated. If these plants are subsequently placed under favorable light and other environmental conditions, they proceed to flower normally. The chain of chemical reactions may be upset at various points if the plants are given an insufficient number of short photoinductive periods or subsequent long photoinductive treatments of 19 or 24 hours of light. This indicates that a complex interacting system, perhaps hormonal in nature, is operative. Disruption at any place in such a chain of reactions as a consequence of the influence of an unfavorable photoperiod causes a variety of morphological and cytological abnormalities.

Summary

1. Experimental groups of *Cosmos sulphureus* Cav. var. Klondike were given photoinductive cycles of 8 hours of light and 16 hours of darkness for 6, 7, 8, 10, 12, and 14 days. The groups were then divided into three lots each, which were given differential postinductive photoperiods of 14, 19, or 24 hours.

2. Controls were run on natural and 8-hour photoperiods; both of these lots flowered profusely. Three other controls

run continuously on 14-, 19-, and 24-hour photoperiods did not flower.

3. Flower buds were collected and fixed successively from all groups. These provided a normal series for floral and meiotic development through pollen formation and material for the study of abnormalities induced by the experimental photoperiods.

4. The markedly suppressed buds of *Cosmos*, usually surrounded by long foliaceous outer bracts and inner inhibited ones, showed no differentiation of floral parts or sporogenous tissue. Less retarded reproductive structures produced floral tube, sporangial wall, a black-line tapetum, and sporogenous cells with little or no cytoplasm. These sporocytes did not initiate the prophase.

5. In plants in which retardation was less marked, various abnormalities occurred, such as degeneration of tapetum, plasmodial masses derived from sporogenous tissues accompanying the normal stages, lagging of chromosomes and the failure of some of them to be included in the microspores, and degenerating cytoplasm in the microspores.

6. No pollen which appeared to be morphologically normal occurred in the anthers of flowers in which tapetal cells did not form a locular matrix.

7. Although a quantitative record of buds of the experimental lots clearly shows a reduction in total reproductive activity in relation to reduced photoinduction and to increased length of the postinductive photoperiod, it is cytologically impossible to draw lines of demarcation between the lots.

The writer wishes to express appreciation to Dr. J. M. BEAL for guidance and suggestions throughout the course of this investigation.

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PHOTOPERIODIC RESPONSES OF GEOGRAPHICAL STRAINS OF ANDROPOGON SCOPARIUS¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 591

EUGENE C. LARSEN

Introduction

Andropogon scoparius Michx. was chosen for investigation because of its extremely wide distribution and recog-

¹ A report of an investigation carried on at the University of Chicago at the suggestion and under the supervision of Dr. CHARLES E. OLMSTED as one in the series "Growth and development in range grasses." This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

nized diversity (6, 7, 10, 12, 16, 22) and because of its importance as a forage grass in many parts of the United States. It has recently been used in the reseeded of abandoned, cultivated land, especially in the central prairie region (8, 9, 11, 15, 21). It is also quite well adjusted to growth in greenhouses during the hot summer months in Chicago.

Information on the photoperiodic re-

sponses of *A. scoparius* should be of value in analyzing the noticeable variations among the ecotypes when they are grown together under greenhouse or nursery conditions (10) and in understanding and explaining the reproductive and vegetative habits of the species. Such information may be of use in varietal selection and in the interpretation of growth and flowering habits of strains grown outside their native habitats. It should also be of interest to those studying the evolution and natural selection of adaptive physiological responses and their relation to plant migration.

There has been relatively little work on photoperiodic responses of various strains of native forage and range plants, although the literature in the general field of photoperiodism is extensive. The latter has recently been summarized by HAMNER (13), WHYTE (23), and BORTHWICK (5). OLMSTED (19) has referred to general literature related to photoperiodic work on grasses.

A. scoparius is surpassed by few species of native American plants in its wide range of habitat and distribution. It has, according to HITCHCOCK (14), been collected in all the states except Washington, Oregon, Nevada, and California. From the standpoint of abundance, its center of distribution is in the tall-grass prairie region between Texas and the Canadian line. Here it was one of the species which made up the major part of the native vegetation of the original prairie.

Its value as a forage plant varies considerably, both with season and with locality. It is generally palatable from the time it starts to grow in the spring until it begins to mature. ROGLER (20) listed *A. furcatus* as the most palatable of all the important warm-season grasses of the prairie states and stated that *A. scopari-*

us was its equal until around August 10, when the culms began to mature. *A. scoparius* was found to be as palatable as any grass on the range in North Dakota during the period from July 12 to 18. It does not withstand heavy spring grazing.

The range of morphological variation in this species complex has led to the recognition of several intergrading forms and varieties (12, 14, 16). HUBBARD (16) stated that the variations may be in color of the plant, length of the sessile spikelet, villousness of the sheath and leaves, compression of the sheath, or length of the hairs at the internodes of the rachis. Some of the described varieties and specific segregates have fairly well-marked geographical ranges.

Specimens of the twelve collections used in this study were sent to the National Herbarium in Washington, D.C., for identification. Dr. J. R. SWALLEN, associate curator, pointed out that strain 2 differs slightly from the others in that it has a broad, more sharply keeled lower sheath which is villous and that the staminate or pedicellate spikelets are about as large as the sessile, fertile ones. He thought that this strain is probably *A. divergens*, a species closely allied to *A. scoparius* and sometimes listed as a variety of it. He considered the eleven other collections to be *A. scoparius* and, although recognizing variation among them, did not assign varietal rank.

No cytological observations have been made upon the plants used in the present experiment. Cytotaxonomic studies (6, 7, 17) of the species and some of its named varieties have shown most of the investigated individuals to be tetraploid plants ($2n = 40$), although differences in structural details of the chromosome complements are correlated with some of the observed morphological variations.

Several workers have contributed to

our recent knowledge of physiological variation exhibited by various ecotypes in grasses. NIELSEN (18), for *Panicum virgatum*, has shown what a complex of diversity one is likely to find when dealing with a large number of ecotypes from widely separated areas. OLMSTED (19), for side-oats grama, found that practically the entire range of reported photoperiodic responses was exhibited by the different ecotypes. He stated that this species cannot be classified photoperiodically except with reference to strains.

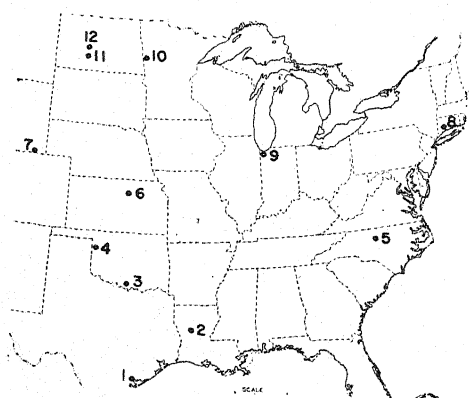


FIG. 1.—Geographical sources of strains numbered as shown.

CORNELIUS (10) has recently described the effects of source of seed of *A. scoparius* on growth, adaptation, and its use in revegetation. In his work at Manhattan, Kansas, with sixteen strains of *A. scoparius* collected from widely separated geographical areas, he reported a difference of 126 days between the first flowering dates of the northern and southern ecotypes. His northern types were earlier in maturity and lower in forage production than the southern ones. The plants originating in central latitudes were intermediate. He obtained a high seed set in the latter plants. The northern ones suffered from hot winds, and the southern ones from frost injury.

He found a highly significant correlation between the flowering date and the dry weight of a plant at the close of the season—early flowering led to low total yield. Winter injury was observed among his southern ecotypes.

The present experiment on photoperiodic responses of geographical strains of *A. scoparius* was initiated in the spring of 1946 and continued through the spring of 1947 in the greenhouses and experimental garden at the University of Chicago.

Material and methods

Forty plants, representing twelve points of origin (fig. 1) were assembled at the University of Chicago in March and April, 1946, through the courtesy of the collectors listed in table 1, and were assigned the indicated designations. Each plant was assumed to be, and apparently was, an individual clone.

On May 1 each of the forty clones was divided, and the divisions, after being trimmed to approximately equal size, were planted in 8-inch, unglazed clay pots. Three segments of a clone were planted in each of five pots to make five identical series of forty pots each.

The pots were filled with soil consisting of a mixture of six parts of coarse sand with five parts of black loam garden soil. No fertilizer was added. This light, sandy-textured soil was used to emulate as nearly as possible the soils found in the native habitats of many of the plants.

One series (*N*) was placed on a greenhouse bench, and another (*G*), after being potted for a 10-day period, was transplanted to an experimental garden. Series *N* and *G* received Chicago natural daylength (interval between sunrise and sunset on June 21 is 15 hours and 14 minutes). The other three series were placed on movable trucks which were

wheeled into separate lightproof, ventilated sheds at 5:00 P.M. and out again at 8:00 A.M. They thus received 9 hours of natural light per day. Supplementary illumination to extend their light periods was supplied by means of three 200-watt incandescent-filament lamps mounted in individual reflectors over each of the trucks. These lights, controlled automatically, were turned on at 5:00 P.M. and were shut off at the hour necessary to provide total consecutive photoperiods of 13, 14, and 15 hours for the three series. Intensity of supplementary illumination at average foliage height

ranged from 100 to 180 foot-candles, depending on the height of a plant and its position on the truck.

Each series was so arranged that the taller plants of southern origin did not shade the smaller northern ones. As they developed, many of the taller culms were staked and tied to prevent them from falling over, breaking off, or shading surrounding plants. Pots were watered adequately to prevent temporary wilting even during hot sunny weather. Sow bugs and insect pests were controlled by the usual methods, and there were no fungus diseases of importance.

TABLE 1
SOURCE OF TWELVE STRAINS OF ANDROPOGON SCOPARIUS

Strain and clone	Point of origin of clone or parental seed lot	Approximate latitude	Clone secured from	Sent by	Accession number of agency
12A	Price, N.D.	47° 10'	Northern Great Plains Field Station, Mandan, N.D.	George A. Rogler, BPI, Mandan, N.D.	D-139-3
11B, C	Mandan, N.D.	46° 50'	Northern Great Plains Field Station, Mandan, N.D.	George A. Rogler, BPI, Mandan, N.D.	D-138-3 (B) D-137-4 (C)
10A, B, C, D, E . .	Moorhead, Minn.	47° 00'	Field	J. J. Westfall, Moorhead, Minn.	
9A, B, C, D, E . .	Dunes State Park, Ind.	41° 30'	Field	E. C. Larsen	
8A, B, C, D	North Haven, Conn.	41° 23'	Field	H. J. Lutz, Yale University, New Haven, Conn.	
7B	Cheyenne, Wyo.	41° 05'	Northern Great Plains Field Station, Mandan, N.D.	George A. Rogler, BPI, Mandan, N.D.	D-6-11
6B, C	Manhattan, Kan.	39° 10'	SCS Nursery, Manhattan, Kan.	M. D. Atkins, SCS, Manhattan, Kan.	KG-1580*(B) KG-480 (C)
5A, B, C, D, E . .	Durham, N.C.	36° 00'	Field, Duke Forest	H. J. Oosting, Duke University, Durham, N.C.	
4A, B, C, D	Arnett, Okla.	36° 10'	Field	James E. Smith, Jr., SCS, Woodward, Okla.	
3A	Ardmore, Okla.	34° 10'	SCS Nursery, Manhattan, Kan.	M. D. Atkins, SCS, Manhattan, Kan.	KG-753
2A, B, C, D, E . .	Alexandria, La.	31° 15'	Field	J. T. Cassady, U.S. Forest Service, Alexandria, La.	
1A, B, C, D, E . .	Matagorda Bay, Jackson County, Texas	28° 15'	SCS Nursery, Tuscon, Ariz.	L. P. Hamilton, SCS, Tuscon, Ariz.	A-2966†

* Improved selection from KG-480.

† Grown from seed from San Antonio, Texas, collection SA-4437.

Observations on culm elongation and flowering were made twice a week or oftener. Heights of the three longest tillers (to tip of outstretched leaf or inflorescence) on each plant were measured on the first and fifteenth day of each month. The three values were averaged.

On November 1, 1946, all plants were harvested by clipping just above the soil

been taken, dry weights of tops were obtained by the usual methods.

The *N* series was then placed back on the greenhouse benches, where most of the plants remained dormant during the winter months in spite of the warm temperatures. The plants in the garden (series *G*), after clipping on November 1, were left there to test their survival over



FIG. 2.—Twelve strains of *A. scoparius* grown on photoperiods of 13 hours. Left to right, below, strains 1, 2, 3, 4, 5, and 6; above, strains 7, 8, 9, 10, 11, and 12. This photoperiod was not long enough to induce internodal elongation. Southern strains show greatest leaf elongation. (Photographed September 1, 1946.)

line. Data were obtained on the total numbers of elongated and unelongated tillers. The central culm of each plant was analyzed for total length and for numbers of visible leaves, nodes, elongated internodes, and flowering branches. If inflorescences were not visible, the growing points were dissected to show whether flowers or flower primordia were present. After these measurements had

winter at Chicago. Strains 1, 2, and 5 winter-killed in the garden. All other plants survived out-of-doors.

Results

FLOWERING RESPONSES

The date of first flowering of a strain in any series was recorded as the date on which anthers were first exerted (first anthesis) by the first clone to flower. The

date of last flowering of a strain is defined as that on which the last-formed inflorescence in its clonal population came into anthesis. The length of flowering season of a strain was measured as the interval between these two extreme dates.

None of the twelve strains flowered on 13-hour photoperiod (table 2, fig. 2).

Plants of only strains 2, 3, 4, and 5 produced visible inflorescences on the 14-hour light period (fig. 3), although on November 1, at the time of harvest, well-developed floral primordia were present in strain 1. Strains 6-12 neither flowered nor showed elongation on 14-hour photoperiod. In the 15-hour series, strains 4 and 6-12 produced flowers (fig. 4), but

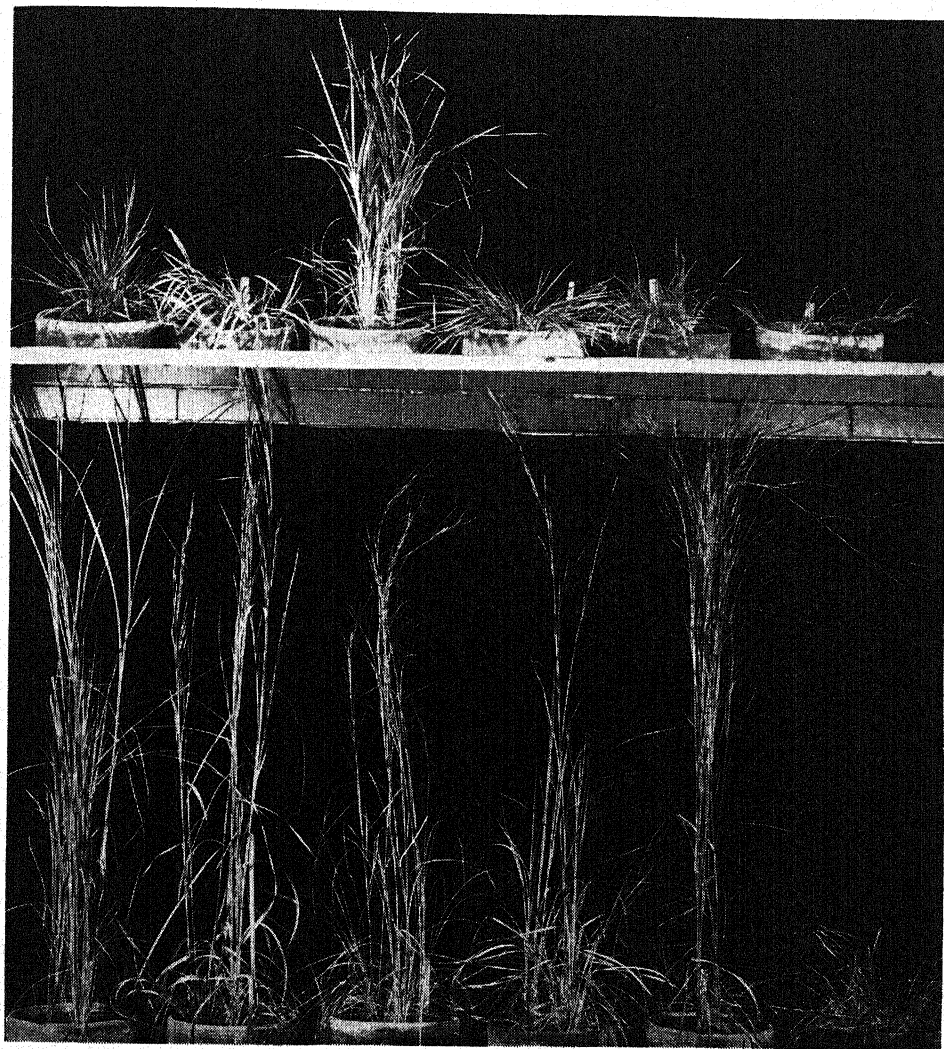


FIG. 3.—Twelve strains of *A. scoparius* grown on photoperiods of 14 hours. *Left to right, below*, strains 1, 2, 3, 4, 5, and 6; *above*, strains 7, 8, 9, 10, 11, and 12. Southern strains 1-5 showed culm elongation and flowering. Strains 6-12 remained vegetative. Strain 9 from sand dunes of Lake Michigan showed greatest vegetative growth among central and northern strains. (Photographed September 1, 1946.)

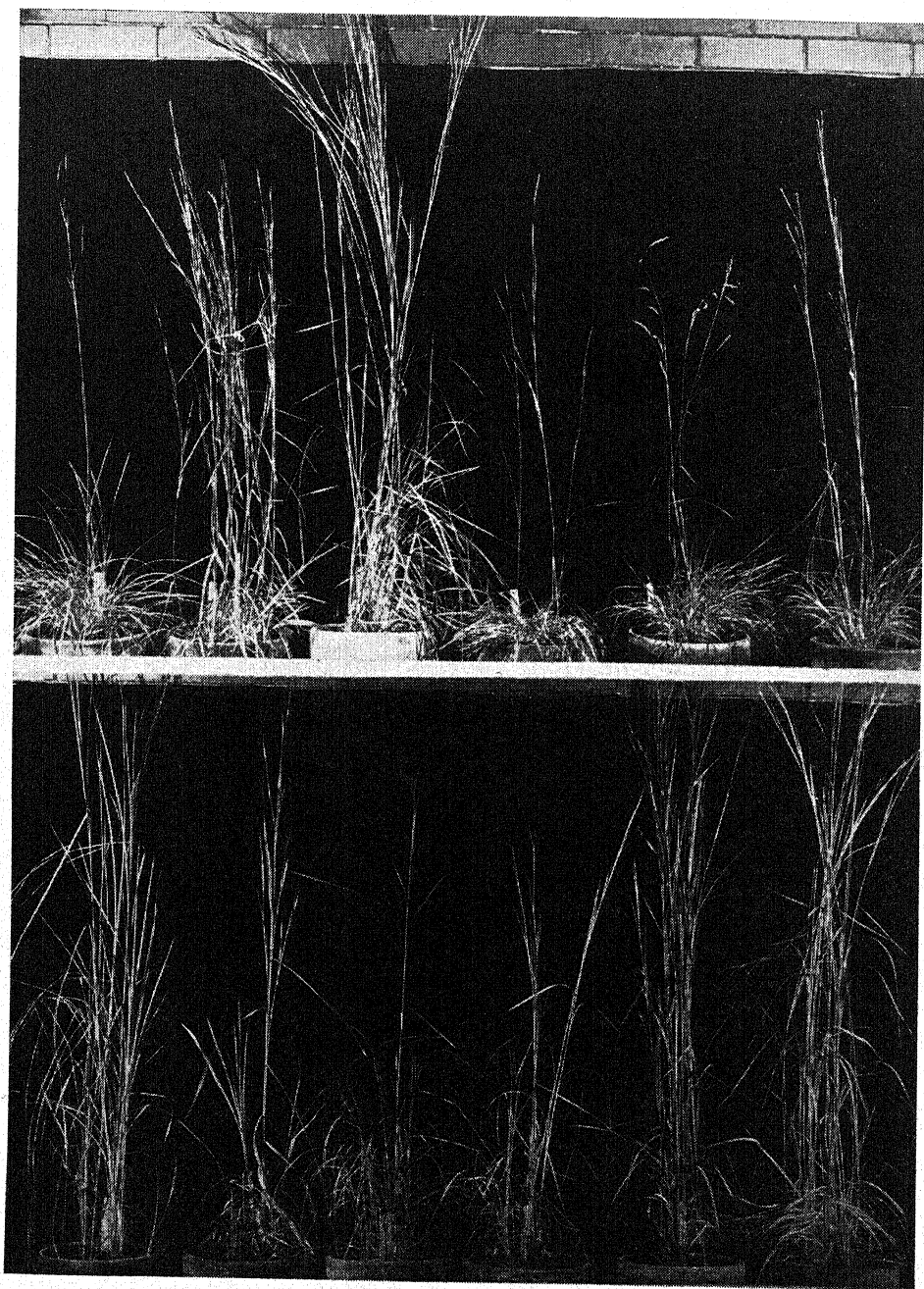


FIG. 4.—Twelve strains of *A. scoparius* grown on photoperiods of 15 hours. Left to right, below, strains 1, 2, 3, 4, 5, and 6; above, strains 7, 8, 9, 10, 11, and 12. All strains with elongated culms. Strains 1, 2, 3, and 5 did not produce flowers by time of harvesting. All plants were still green on harvesting date, November 1. (Photographed September 1, 1946.)

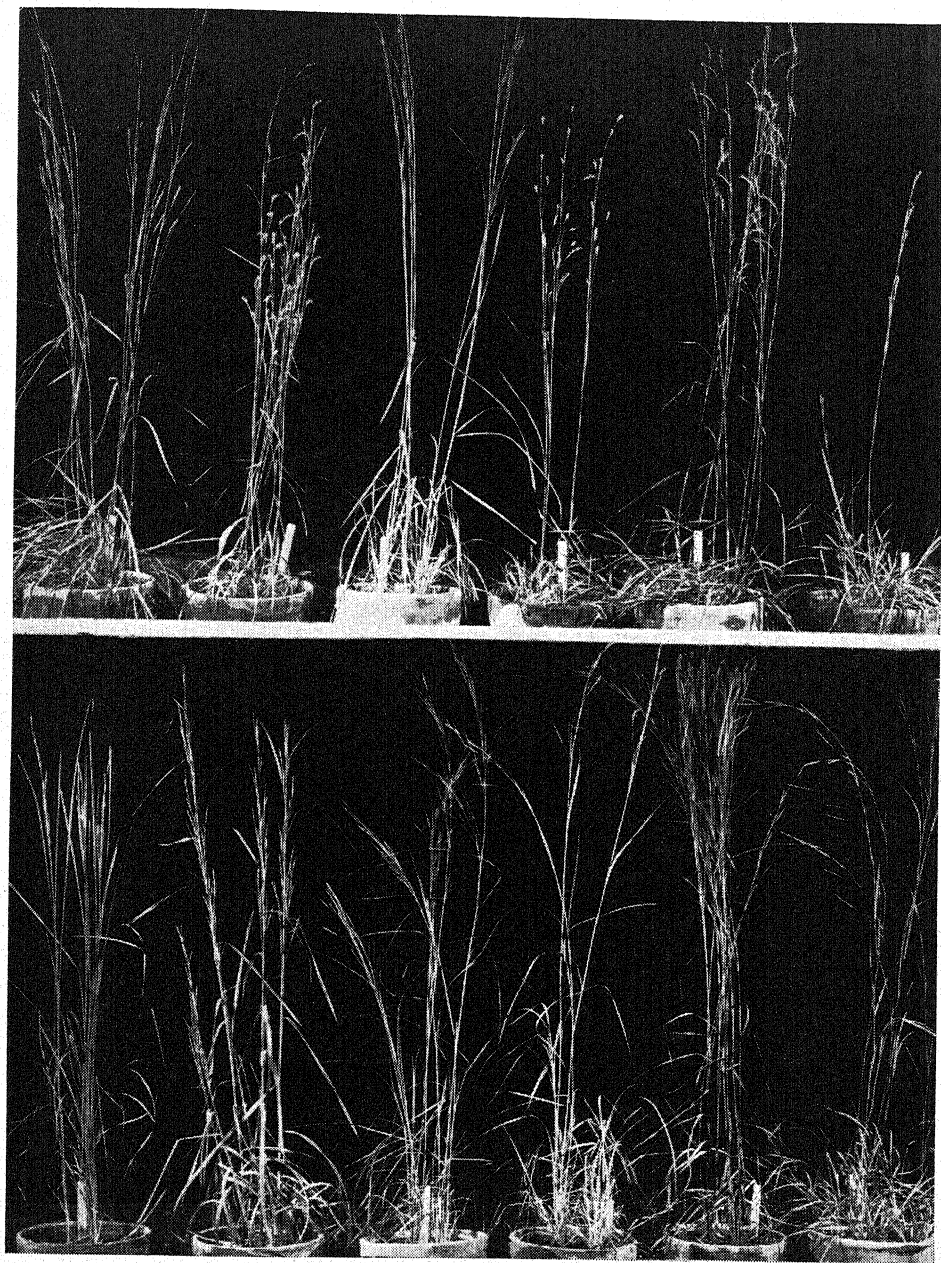


FIG 5.—Twelve strains of *A. scoparius* grown on Chicago natural daylength in the greenhouse (*N* series). Left to right, below, strains 1, 2, 3, 4, 5, and 6; above; strains 7, 8, 9, 10, 11, and 12. All strains flowered except strain 6. Northern strains have begun to enter dormancy. (Photographed September 1, 1946.)

the southern strains 1, 2, 3, and 5 had not visibly flowered at harvest time, even though culms were elongated. Dissection of their growing points showed that three of fifteen plants in strain 2, and five of ten in strain 5, had poorly developed floral primordia. Representatives of all strains flowered in the *N* series (fig. 5) except strain 6, which showed a low percentage of elongated culms. In the *G* series all clones flowered except 2C, 2E, 6C, and 10D. Plants 2E and 10D did not flower in any series.

TIME TO FIRST FLOWERING.—Table 2 shows the number of days after clonal division and planting on May 1 until first anthesis in each strain in each series. There was a tendency in any one treatment for southern strains to be delayed in flowering in comparison with northern ones. This tendency was most consistent in series *G*, in which all clones were most vigorous and in which most clones of all strains flowered. In this series strain 1 from Texas came into flower 65 days later than strain 12 from North Dakota.

In five out of the seven strains flowering in both the *N* and the 15-hour series, flowering was earlier on natural daylength. These data suggest a delay in the time of first flowering in these strains on the 15-hour photoperiod. This was brought out more clearly when the comparison of the two series was based on the flowering data of individual plants rather than on the earliest representatives in each strain. There was no consistent tendency for the 14-hour photoperiod either to delay or to accelerate flowering in comparison with the *N* series for the five southern strains flowering on each. In nine strains it required a longer time to first flowering in the *G* series than in the *N* series. In strain 2 the two series flowered at the same time. In strain 12 series *G* flowered first, and there was no

flowering in strain 6 in series *N*. It is possible that the delay in series *G* in comparison with *N* may have been related to the average cooler temperature conditions in the garden, which delayed early growth in series *G* and also could have lengthened the time between floral initiation and anthesis.

FLOWERING SEASON.—Extremes in first- and last-flowering dates of each strain and the intervals between are also shown in table 2. One is impressed with the variability among the strains in the length of flowering season in any one treatment. This showed no correlation with latitude. There was also a noticeable tendency for the flowering season of a strain on 14- or 15-hour photoperiod to be longer than in the *N* or *G* series. This was more obvious and significant when the comparison was based on individual plants rather than on those representing the extreme dates in a strain. The data suggest that in some cases the flowering season was lengthened by constant photoperiod or was shortened by the progressively decreasing daylength.

The above suggestion is borne out by the fact that, with the coming of autumn, a state of dormancy was initiated in the *N* and *G* series in which vegetative and reproductive activity ceased in all but the most southern strains, even though the autumn was very mild and frost injury to plants of series *G* was not evident until the middle of November. The plants on 13-, 14-, and 15-hour photoperiods remained green, and those in the 15-hour series continued to produce new elongated tillers. The length of the flowering seasons of some 14- and 15-hour plants was limited by harvesting.

INTERNODAL ELONGATION.—The time required after May 1 for evident elongation of internodes is shown in table 3. Such elongation is sometimes taken as

a criterion of the onset of reproductive activity, but, as shown later, this is not valid in *A. scoparius*. Differences between series in any one strain were probably not significant, except where elongation was completely inhibited on 13- or 14-hour photoperiods. The greatest range between strains was

in series G, in which strain 8 showed elongation in 68 days; strain 1, in 132 days. In general, it required longer for the southern strains to show elongation than the northern ones.

The length of time between first evident elongation and first flowering (table 3) was more positively correlated with

TABLE 2
FLOWERING SEASON*

STRAIN NO.	No. OF DAYS AFTER CLONAL DIVISION ON MAY 1 UNTIL FIRST FLOWERING				No. OF DAYS BETWEEN FIRST AND LAST FLOWERING			
	Photoperiod				Photoperiod			
	14	15	N	G	14	15	N	G
12.....		110	124	114		48	1	37
11.....		121	101	124		16	23	9
10.....		121	110	119		58	4	18
9.....		139	121	132		33	30	33
8.....		105	110	129		60	48	22
7.....		144	114	124		1	18	21
6.....		151		144		28		28
5.....	124		144	148	41		35	24
4.....	147	151	132	145	32†	28†	19	20
3.....	132		139	151	40		12	14
2.....	140		144	144	32		14	28
1.....	†		172	179	†		7	†
FIRST AND LAST DATES OF FLOWERING								
Photoperiod								
14		15		N		G		
First	Last	First	Last	First	Last	First	Last	
12.....			8-18	10-5	9-1	9-1	8-22	9-28
11.....			8-29	9-14	8-9	9-1	9-1	9-9
10.....			8-29	10-26	8-18	8-22	8-27	9-14
9.....			9-16	10-19	8-29	9-28	9-9	10-12
8.....			8-13	10-12	8-18	10-5	9-6	9-28
7.....			9-21	9-21	8-22	9-9	9-1	9-21
6.....			9-28	10-26			9-21	10-19
5.....	9-1	10-12			9-21	10-26	9-25	10-19
4.....	9-24	10-26†	9-28	10-26†	9-9	9-28	9-22	10-12
3.....	9-9	10-19			9-16	9-28	9-28	10-12
2.....	9-17	10-19			9-21	10-5	9-21	10-19
1.....	†				10-19	10-26	10-26	†

* No plants flowered on 13-hour photoperiod.

† Still flowering when harvested.

‡ Floral primordia present on November 1—184 days after clonal division.

strain and treatment. Generally the southern clones required a longer interval than the northern ones in any one series. The number of days between elongation and flowering in general was greater in the 15-hour series than in the *N* series for plants flowering in each. Strain 4 shows the delay on 15 hours quite clearly. In the 14-hour and *N* series it flowered 37 days after initial elongation; in series *G*,

ably be classed as intermediate-day plants rather than short-day, since they failed to flower on 13-hour photoperiod, flowered well on 14-hour photoperiod, and failed to flower or were delayed in flowering on 15-hour photoperiod.

In summary, these data on flowering strongly suggest that (a) the five southern strains are intermediate-day plants in their photoperiodic responses and that

TABLE 3
INTERNODAL ELONGATION*

STRAIN NO.	LENGTH OF TIME TO FIRST EVIDENT ELONGATION AFTER MAY 1				LENGTH OF TIME BETWEEN FIRST EVIDENCE OF ELONGATION AND FIRST FLOWERING			
	Photoperiod				Photoperiod			
	14	15	<i>N</i>	<i>G</i>	14	15	<i>N</i>	<i>G</i>
12.....		83	110	99		27	14	15
11.....		100	87	107		21	14	17
10.....		111	95	107		10	15	12
9.....		90	95	99		49	26	33
8.....		76	83	68		29	27	61
7.....		114	99	107		30	15	17
6.....		110	124	114		41		30
5.....	76	76	76	99	48		68	49
4.....	110	95	95	114	37	56	37	31
3.....	100	114	95	114	32		44	37
2.....	100	124	104	99	40		40	45
1.....	104	104	104	132	†		68	47

* No evident internodal elongation in 13-hour series.

† Floral primordia were present 80 days after evidence of elongation.

in 31 days. On 15-hour photoperiod the interval was 56 days. Such a delay in strain 4 in the 15-hour series and the failure of strains 1, 2, 3, and 5 to flower visibly on this photoperiod suggest that these southern strains have an upper critical limit for flowering at around 15 hours or would be much delayed in flowering on photoperiods of this or greater lengths. It may be recalled that a few culms on some plants of strains 2 and 5 on 15-hour photoperiod showed floral primordia when dissected on November 1. Accordingly, strains 1-5 should prob-

(b) the northern strains may be long-day plants, since they are able to initiate flowers on the long natural photoperiods of June² and July in Chicago and flowered on 15-hour constant photoperiod but were inhibited from flowering on 13- and 14-hour photoperiods.

FLOWERING BRANCHES.—A flowering shoot in *A. scoparius* usually includes one or more racemes in a terminal inflorescence and four to six or more axillary flowering branches arising from the upper nodes of the culm. The average num-

² As shown by experiments carried on in 1947.

ber of such branches on the central culm at final harvest is shown in table 4. In individual cases they had been produced at nearly all the nodes of the elongated culm. In strain 5 even the axillary buds in the lower leaf sheaths often developed into flowering branches. In the other strains these lower buds usually failed to develop.

The number of axillary buds that developed into flowering branches varied with the geographical strains. In general, the southern and East Coast strains produced a greater number of such branches per culm than the northern and western ones. Strains from the extreme North and the extreme South failed to produce as many as the more centrally located strains.

There was a somewhat direct relationship between the height and number of nodes of a culm and the number of flowering branches. The plants in the garden produced more flowering branches than those on any other treatment. In six of the seven strains which flowered in both the *N* and the 15-hour series, those plants on 15-hour photoperiod produced fewer flowering branches than those in the *N* series.

VEGETATIVE RESPONSES

TILLER NUMBER AND ELONGATION.—The most striking effect of photoperiodic treatment was upon the degree of elongation of leaf blades and sheaths and internodes of stems. There apparently is a distinct critical photoperiod above which plants of a strain showed marked elongation of culms and leaves and below which such elongation was exceedingly limited. None of the plants on 13-hour photoperiod showed such elongation; all of them on 15-hour photoperiod did so (table 5, figs. 2-4). The critical photoperiod for elongation in the species is ap-

parently between 13 and 15 hours. On 14-hour photoperiod, plants of the southern strains (1-5) elongated and produced flowers, while those of northern strains (6-12) showed no elongation. Strain 5 originated at latitude 36° N., strain 6 at 39° N.

The plants on 13-hour photoperiod were much like those on other treatments in growth habits for a few weeks after planting. Their rate of height growth

TABLE 4
AVERAGE NUMBER OF FLOWERING BRANCHES
PER CENTRAL CULM*

STRAIN NO.	PHOTOPERIOD			
	14	15	<i>N</i>	<i>G</i>
12.....	3	4	6
11.....	5	6	5
10.....	2	5	5
9.....	5	4	8
8.....	7	8	11
7.....	2	5	4
6.....	6	9
5.....	10	12	13
4.....	5	6	6	6
3.....	8	8	14
2.....	7	7	14
1.....	5	7

* Based on flowering plants only.

then diminished rapidly, and they showed little increase in height through the remainder of the season. The southern strains grew more rapidly than the northern ones at first and continued to grow for several weeks after the northern ones appeared static. The latter showed little increase in height after the first 2-4 weeks on 13-hour photoperiod, even though new leaves continued to form. However, the blades and sheaths and internodes failed to elongate normally. Large buds formed in the axils of the leaf sheaths. The expansion of these buds forced the leaf sheaths open, spreading the two-ranked leaves apart. Some of the

fanlike rosettes approached a horizontal plane, but more of them oriented themselves at right angles to the rays of the sun (fig. 6).

Individual plants which failed to show elongation all produced large numbers of tillers. Apparently conditions which hinder elongation or floral initiation may favor an increase in total tiller number. In general, the plants which did not

from each plant, and the total number of leaves on it was recorded (table 6). In some strains there was no obvious correlation between number and photoperiod or the amount of culm elongation. In part this may have resulted from the difficulties in making accurate counts of the leaves on the unelongated culms in the 13- and 14-hour series. Strains 7, 9, and 10, in which these difficulties were

TABLE 5
TILLER NUMBER

STRAIN NO.	AVERAGE NO. OF TILLERS PER PLANT ON NOVEMBER 1					PERCENTAGE OF TOTAL NO. OF TILLERS THAT ELONGATED*			
	Photoperiod					Photoperiod			
	13	14	15	N	G	14	15	N	G
12.....	24	11	17	9	40	35	26	29
11.....	11	18	21	12	33	20	50	11
10.....	7	10	10	8	31	27	16	9
9.....	9†	9†	9	6	39	59	44	45
8.....	18	14	11	13	14	39	22	51
7.....	7†	9†	11	15	38	35	63	17
6.....	27	25	12	32	93	66	8†	27
5.....	21	11	7	11	14	26	53	27	46
4.....	21	17	9	19	38	30	58	24	18
3.....	26	12	9	16	24	27	40	28	58
2.....	10	7	5	14	13	43	33	16	62
1.....	9	7	7	8	27	42	44	26	72

* No elongation on 13-hour photoperiod.

† Large buds not counted as tillers.

‡ Plant did not flower.

flower produced more tillers than those that did. The 15-hour plants, when taken as a whole, produced the smallest number of tillers of all the treatments.

At final harvest, ten of the strains showed a higher percentage of tillers elongated on 15-hour photoperiod than in the N series. The excepted strains are strain 7, in which insect injury to plants on 15-hour photoperiod probably retarded elongation, and strain 11, one clone of which did not flower in this series.

NUMBER OF LEAVES.—At the time of harvest, the central culm was removed

not great, definitely had more leaves per culm under the 13-hour treatment, with no elongation, than in the other series. In contrast, in the southern strains 1, 2, 3, and 4, the numbers were lower in the plants on 13-hour treatment in which no elongation occurred than in the same clones in the other series. These southern strains, however, were much taller in the 13-hour series than were the other strains which produced more leaves per central culm. In the other series, in general, the southern strains produced more leaves per culm than the northern ones. Strain

5 from North Carolina averaged twenty-one leaves on each central culm in series *N*.

GROWTH IN HEIGHT.—Height measurements taken periodically through the summer of 1946 showed that, on 13-hour photoperiod, strains 1-4 reached their maximum height before July 1. On 14-hour photoperiod, strains 1-2 grew in height until October 15; strains 3-5 continued to increase until about September 15 to October 1, while strains 6-12 showed little change in height after July 1. In the other series in the plants which flowered, growth curves tended to begin to level off just prior to first anthesis, and most of these plants reached their maximum height at about the time of full anthesis.

At harvest time the central culm from each pot was clipped at ground level and was measured from the cut end to the tip of the outstretched leaf or inflorescence. The averaged values are given in table 6. The flowering plants were taller than the nonflowering. The shortest plants were in the northern strains 5-12 on 13-hour

photoperiod. Southern strains 1-4 in this series were taller, even though they failed to show marked internodal elongation or to flower. On 14-hour photoperiod, strains 1-5 were very tall, but strains 6-12 were about the same height as on 13-hour photoperiod (figs. 2, 3). In general, all strains were much taller in the 15-hour, *N*, and *G* series than on 13-hour

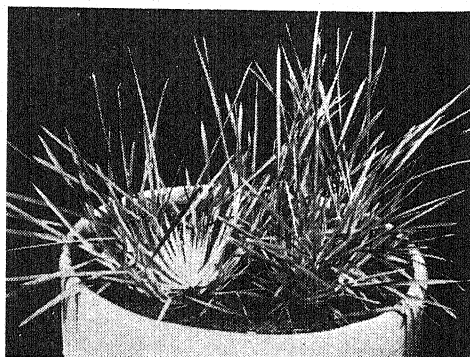


FIG. 6.—Strain 12 from North Dakota; characteristic rosette condition developed in northern strains grown on 13-hour photoperiod. Leaves continued to form even though blades, sheaths, and culms failed to elongate normally. (Photographed September 29, 1946.)

TABLE 6
CENTRAL CULMS; DATA OF NOVEMBER 1

STRAIN NO.	AVERAGE NO. OF VISIBLE LEAVES					AVERAGE MAXIMUM HEIGHT (CM.)				
	Photoperiod					Photoperiod				
	13	14	15	<i>N</i>	<i>G</i>	13	14	15	<i>N</i>	<i>G</i>
12.....	8.5	11.1	8.5	9.7	8.0	18	15	103	58	78
11.....	8.8	8.0	10.4	11.2	7.5	21	24	52	109	68
10.....	11.5	9.3	10.3	7.3	4.9	18	22	47	47	44
9.....	17.3	12.4	11.6	11.3	11.9	36	49	93	77	95
8.....	12.0	9.3	11.6	12.0	13.0	23	32	70	77	95
7.....	18.7	9.0	9.7	10.0	7.3	18	31	90	108	60
6.....	8.7	7.3	13.2	7.5	11.0	39	30	107	43†	69
5.....	11.2	13.2	18.3	21.3	17.3	26	107	100	121	98
4.....	7.3	11.5	13.0	13.1	10.2	49	69	97	112	55
3.....	8.0	12.8	10.3	15.5	17.0	63	100	53*	108	95
2.....	8.3	15.4	15.9	13.3	16.4	55	106	86	103	77
1.....	8.0	15.8	11.8	13.8	14.3	82	119	110	99	89

* Plant weak.

† Plants failed to flower.

photoperiod. The various clones of a strain and series in the greenhouse were more variable in height than in the garden.

TOP WEIGHT.—At harvest time the tops were clipped just above soil level, and oven-dry weights were determined (table 7). In all strains except 7 and 11, plants of series *G* yielded more than the comparable plants in any of the other four series. This probably was related in

however, produced more dry weight than any of the others in series *G* and stood in second or third rank in the 13-, 14-, and 15-hour series. Apparently this strain, native to open sterile dune sand, is able to respond vigorously to improved soil and water relations.

In many strains the effect of photoperiod upon dry weight was striking. Strains 1 and 2 yielded more in the 14-hour series than in the 13-hour, 15-hour, or *N* series. Many of the northern strains produced only about half as much weight on 13-hour photoperiod as they did on 15 hours. They are usually dormant under natural conditions when day-lengths are 13 hours or lower. Some of them also yielded more on the 15-hour treatment than in the corresponding *N* series. This may be correlated with the effect of the former in inducing continued vegetative and reproductive activity, while the plants in the *N* series became relatively dormant after flowering on the decreasing natural daylength.

It should be emphasized that the differences in dry-weight yield among the 13-, 14-, and 15-hour series are an expression of photoperiodic effects and cannot be related to the differences in total irradiation as affecting opportunity for photosynthesis and carbohydrate accumulation. The three series were all exposed equally to 9 hours of natural light per day and differed only in their lengths of exposure (4, 5, or 6 hours) to supplementary illumination of an intensity too low to be very effective in carbohydrate synthesis.

Discussion

The results have shown that *A. scoparius* as a species is strikingly sensitive to photoperiod. It has also demonstrated conclusively that there is considerable differentiation within this widely

TABLE 7
AVERAGE DRY WEIGHT (GM.) PER PLANT OF
CLIPPED TOPS, NOVEMBER 1, 1946

STRAIN NO.	PHOTOPERIOD				
	13	14	15	<i>N</i>	<i>G</i>
12.....	24.7	23.5	49.0	18.5*	113.0
11.....	18.0	20.0	45.9	59.1	52.3
10.....	12.9	17.1	24.8	20.5	37.0
9.....	53.7	57.7	81.8	61.0	344.8
8.....	21.9	26.2	45.0	35.6	92.0
7.....	5.0	39.7	36.0	96.7	83.0
6.....	53.0	22.0	117.4	56.2	231.7
5.....	29.5	57.7	64.6	73.5	123.7
4.....	43.5	52.1	57.8	81.4	91.2
3.....	62.2	47.0	29.5*	80.2	180.0
2.....	52.5	78.9	51.3	67.0	253.4
1.....	55.6	89.2	79.7	55.3	327.8

* Plants weak.

large part to the limiting effect of reduced light intensity in the greenhouse upon the four series grown indoors as well as to the effect of pot culture. The degree of this limitation was most striking in strain 9 from the Indiana sand dunes. However, differences in yield among strains and in relation to photoperiodic treatment were obvious in the greenhouse plants. The southern strains, in general, yielded more than the northern ones in all series. The southern plants are more robust and grow vegetatively for a longer time. In their native habitats they have a longer frost-free period. Strain 9 from Indiana Dunes State Park,

ranging species as to the nature of the photoperiodic response. Somewhat similar differentiation in side-oats grama has previously been reported by OLMSTED (19).

The data for 1946 show that culm elongation, dry-weight yield, and floral initiation are sharply controlled by the photoperiod upon which the plants are grown. There is a critical lower photoperiod below which dry-weight yield is low, culms do not elongate, and floral initiation is lacking. For strains of latitude 36° and southward, this critical photoperiod lies between 13 and 14 hours; for strains of latitude 37° and northward, between 14 and 15 hours. Above this critical photoperiod, all strains showed culm elongation and relatively vigorous vegetative growth. Additional experiments in 1947 indicated that in many strains an 18-hour photoperiod tended to induce earlier culm elongation than did a 15-hour one or than did natural daylength at Chicago.

All these data suggest a long-day status of the species. That this is not true of all the strains, however, is shown by the fact that strains 1, 2, 3, and 5 either failed to flower or were much delayed in flowering on 15-hour photoperiod in comparison with a 14-hour one. For floral initiation and vigorous and rapid flowering, they seem to require a narrow range of photoperiods lying between 13 and 15 hours. They would thus be classed as intermediate-day plants. Similar findings were made by ALLARD (1), by ALLARD and EVANS (2), and by ALLARD and GARNER (3) in related species or genera. CORNELIUS (10) found in his collection of strains of *A. scoparius* grown at Manhattan, Kansas, that the southern ones were delayed in flowering until the short days of autumn and were likely to be injured by frost while still vigorously grow-

ing. In their native latitudes they experience photoperiods favorable to floral initiation early enough to have flowered and entered dormancy before any danger of frost but in Kansas were not exposed to such photoperiods until August or later.

An intermediate-day status for the northern strains (6-12), based on flowering responses, has not been conclusively demonstrated, and they may probably be classed as long-day plants with a critical photoperiod between 14 and 15 hours. One clone each of strains 6-12 flowered on 18-hour photoperiod in 1947 but were somewhat delayed in doing so in comparison with the same clonal divisions on natural daylength or on 15-hour photoperiod. It is very likely that the northern strains flower normally over a wider range of photoperiods than do the southern ones. The time of onset of flowering in them, in July or August, in their native habitats suggests that these northern strains initiate flowers during the season of long natural photoperiods in June and July. The experimental data indicate in part that possibly the natural decreasing photoperiods are more favorable for the later development of inflorescences than are constant long ones. The experimental results also lead one to the conclusion that the decrease in natural daylength below a certain level may be partially responsible for the cessation of vegetative and reproductive activity and the onset of dormancy, especially in the northern strains.

Since the great majority of species of *Andropogon* are tropical or subtropical in distribution, it is very likely that *A. scoparius* originated in low latitudes in which an intermediate- or short-day response would be typical. It is likely that the northward migration of the species into the higher latitudes of the United

States took place along at least two routes: through the central grassland region and up the Atlantic coastal plain.

On the basis of morphological similarities and dissimilarities, it seemed possible to separate the twelve strains used into three groups: northern (strains 7, 10, 11, 12), central (strains 3, 4, 6, 9), and southern or eastern (strains 1, 2, 5, 8). It will be noted by referring to figure 1 that these morphologically differentiated groups are not correlated solely with latitude but with altitude, physiographic provinces, and vegetational regions also, and, for the central and eastern and southern groups, with the suggested routes of migration. It is possible that the groups are genetically differentiated from one another by reason of isolation of environment. Within each group there may have been a greater chance for genetic interchange than between groups. If this hypothesis is correct, it is interesting that two of the three groups include both intermediate- and long-day strains, suggesting the evolution of the long-day response along both of the suggested routes of migration northward. Since the species is a warm-season grass in accord with its probable origin in low latitudes, a northward migration would have been dependent upon the evolution of the long-day or day-neutral habit in the migrating populations, or at least upon an increase in length of the upper critical photoperiod above that now found in strains 1, 2, 3, and 5. Only in this way could the species have become adjusted to flower during the short growing seasons of the northern latitudes while still retaining the high minimum-temperature requirements for growth still characteristic of it.

Summary

1. Forty plants of *Andropogon scoparius* from twelve points of origin were assembled at the University of Chicago

greenhouses in the spring of 1946 and were numbered as shown in figure 1. The twelve geographical strains represented a latitudinal range of approximately 21° from Jackson County, Texas, to Price, North Dakota, and an east-west range from North Haven, Connecticut, to Cheyenne, Wyoming. Their responses to natural daylengths in greenhouse and garden and to constant photoperiods of 13, 14, and 15 hours in the greenhouse were investigated.

2. Growth and development were found to be sharply sensitive to length of photoperiod. None of the twelve strains flowered on 13-hour photoperiod. Plants of only southern strains 2, 3, 4, and 5 produced visible inflorescences on the 14-hour light period. Strain 1 had initiated floral primordia in this series when harvested on November 1. In the 15-hour series, strains 4 and 6-12 produced flowers, but the southern strains 1, 2, 3, and 5 had not visibly flowered at harvest time, even though culms were elongated. Representatives of nearly all the strains produced visible flowers on natural daylength, the southern ones later than the northern. Strains 1-5 should probably be classed as intermediate-day plants rather than short-day, since they failed to flower on 13-hour photoperiod and also failed to flower or were delayed in flowering on 15-hour photoperiod. The northern strains may be long-day plants, since they are able to initiate flowers on long photoperiods but were inhibited from flowering on 13- and 14-hour photoperiods.

3. There apparently is a distinct critical photoperiod above which plants of a strain showed marked elongation of culms and leaves and increase in dry weight and below which such elongation or increase was exceedingly or somewhat limited. None of the plants on 13-hour photoperiod showed elongation; all of

them on 15-hour photoperiod did so. On 14-hour photoperiod, plants of the southern strains (1-5) elongated and produced flowers, while those of northern strains (6-12) showed no elongation. Strain 5 originated at latitude 36° N., strain 6 at 39° N.

4. With the coming of autumn a state of dormancy was initiated in the two series on natural daylength; vegetative and reproductive activity ceased in all but the most southern strains. Plants on 13-, 14-, and 15-hour photoperiods remained green, and those in the 15-hour series continued to produce new elongat-

ed tillers. At final harvest, in general, the strains showed a higher percentage of tillers elongated on 15-hour photoperiod than in the indoor series on natural daylength.

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SOME EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON FRUIT-DROP AND MORPHOLOGY OF ORANGES¹

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Introduction

Recent work has indicated that 2,4-dichlorophenoxyacetic acid (2,4-D) may be superior to other growth-regulating substances for the prevention of preharvest drop of certain varieties of apples (2). As with other compounds of this type, its effectiveness in increasing tomato fruit-set has also been demonstrated (12). In Florida (5) naphthaleneacetic acid and naphthaleneacetamide, when applied in aqueous sprays several months before fruit-drop is expected, have been shown to decrease preharvest drop of pineapple oranges; when applied at the time of fruit-drop, they did not decrease the drop. These sprays have not come into commercial use because growers prefer to risk fruit loss rather than to incur the possibly unnecessary expense of spraying several months in advance of anticipated fruit-drop. Tests with these two chemicals in California were likewise unsuccessful in decreasing preharvest drop of Washington Navel oranges, when applied after the drop had begun. Applications of a wide variety of growth substances also failed to increase fruit-set of Washington Navel oranges or of grapefruit (9). For these reasons, problems of fruit abscission in southern California are again being studied to determine the possible use of 2,4-D and re-

lated compounds in decreasing fruit-drop of oranges.

PERIODS OF FRUIT-DROP.—Abscission of orange fruit is not restricted to the dropping of matured fruit but may occur at any time during fruit development; in fact, it may be considered a continuous process. There are usually three periods, however, during fruit growth, when abscission may be intense. The first is the period of fruit-set, which usually lasts for a month following full bloom. Generally, sufficient fruit remains on the tree during this period so that prevention of abscission is not an acute problem. There are times, however, when it would be desirable to increase fruit-set.

The second period of intense fruit abscission may occur at the onset of hot summer weather and is referred to as "June drop" (3). It is particularly severe with Washington Navel oranges growing in the inland districts of California. At the time of this drop, the young fruit is usually 1.5–2.3 cm. in diameter. Valencia oranges may also lose some of their young fruit during this period, although the drop is less than with Navels.

The third period of intense fruit abscission is called "preharvest drop." This may occur any time after the fruit begins to lose its green color and turn yellow and then orange. With both Valencia and Navel oranges this change in coloring takes place in the late fall, concurrently with the beginning of low night temperatures. Valencia oranges are not picked, however, until the following summer, as

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they do not attain the minimum soluble solids-to-acid ratio of 8 to 1 until that time. It is sometimes even desirable to hold the fruit on the trees for the fall market. Navel oranges, on the other hand, may attain the 8-to-1 ratio by December of the same year in which they flower and are accordingly harvested during the winter. The wet, cloudy weather and severe winds of the winter season hasten the natural fruit-drop of both varieties. Hot summer weather, which increases the June drop of immature fruits, also seems to increase the loss of mature Valencia oranges.

2,4-D were varied from 1 to 240 p.p.m. The higher concentrations used in these tests with 2,4-D (60–240 p.p.m.) were much higher than those employed in previous tests with naphthaleneacetic acid and naphthaleneacetamide in Florida (5), because even the highest concentrations of those two chemicals, in previous tests in California, had failed to decrease fruit abscission. Since the tolerance of orange trees to 2,4-D was not known at the beginning of the present studies, usually only one-quarter or one-half of a tree was sprayed.

FRUIT-SET.—To determine whether

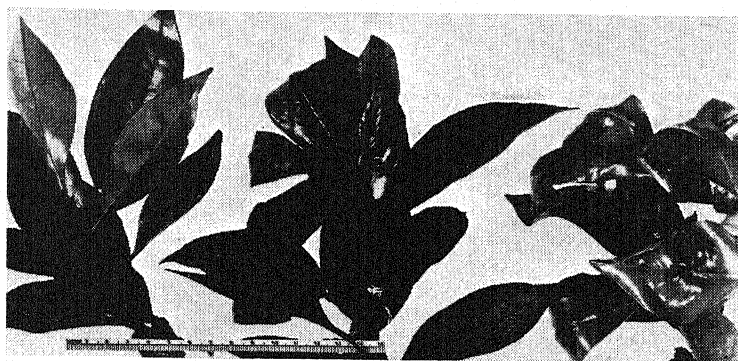


FIG. 1.—Curling of young, expanding Valencia orange leaves in response to aqueous sprays (left to right) of 0, 15, and 30 p.p.m. of 2,4-D. (Photographed 10 days after spraying.)

The present paper reports some of the effects of 2,4-D on fruit abscission during the three periods described above.

Experimentation

All the 2,4-D applications referred to in this paper were made as water sprays, using diethanolammonium 2,4-dichlorophenoxyacetate, which was obtained as a commercial liquid preparation containing the equivalent of 40% free 2,4-D. No supplementary spreading agent was used. The spray equipment varied from 3-gallon, continuous-pressure, Hudson-type garden sprayers to 600-gallon, high-pressure spray rigs. Concentrations of

2,4-D would increase fruit-set, two field plots of Valencia orange trees were established—one plot at East Covina and the other at Glendora, California.

At the East Covina plot, concentrations of 5, 15, 30, and 60 p.p.m. of 2,4-D were tested. At each concentration the spray was applied to the east half of one tree and to the west half of another. Applications were made on April 19, 1946, during the period of full bloom. Mature leaves showed no apparent response, but within 24 hours all treatments had induced a downward and inward rolling of the margins of the young, expanding leaves (fig. 1). The response of the young

leaves was most pronounced at the highest concentrations of 2,4-D and decreased with decreasing concentrations so that it was barely perceptible with the 5-p.p.m. spray. Two to 3 weeks after spraying it became apparent that the treatment had increased the number of

twigs from sprayed trees, the ovary, stamens, and corolla in nearly every flower remained securely attached to the receptacle, whereas on the unsprayed twigs a large percentage of the flowers dropped, and of other flowers only the ovary remained.

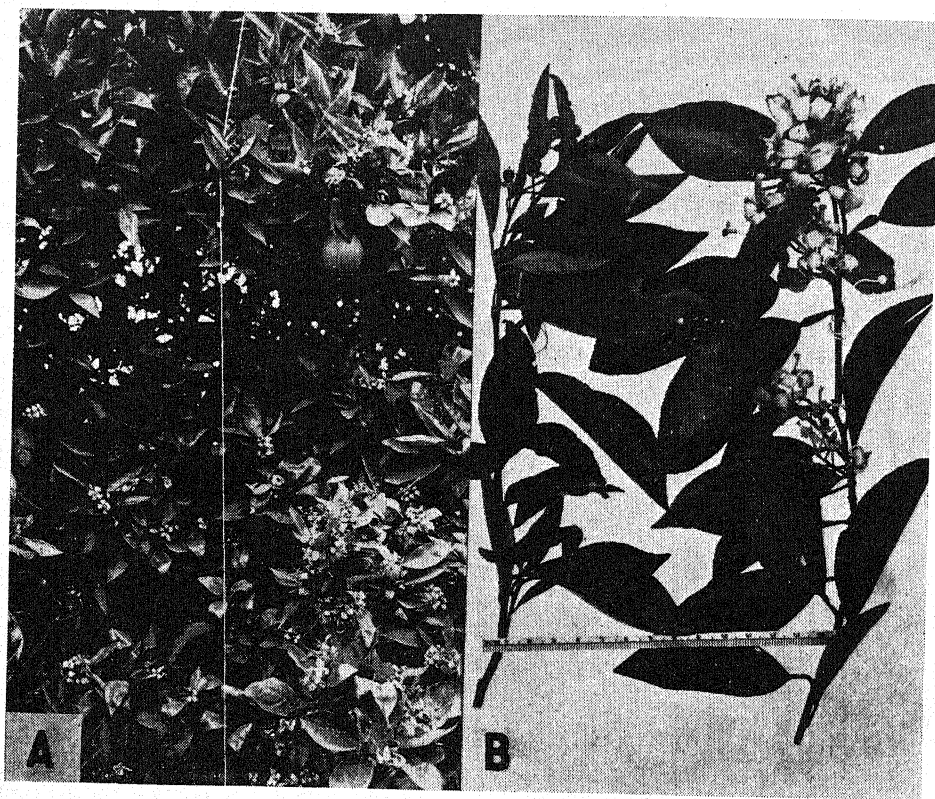


FIG. 2.—Effect of aqueous spray of 60 p.p.m. of 2,4-D applied to Valencia orange tree at full bloom, April 19, 1946. *A*, left, not sprayed; right, sprayed. *B*, representative twigs from unsprayed (left) and sprayed (right) portions of same tree. (Photographed May, 1946.)

flowers retained on the trees (fig. 2, *A*, *B*).

As is well known, ethylene vapors greatly accelerate the rate of abscission of leaves, flowers, and fruits. When twigs from the sprayed and unsprayed trees were exposed to ethylene in the laboratory, they showed that the 2,4-D had within 1 week effectively decreased flower and leaf abscission (fig. 3). On the

In the field the complete flowers were still retained on the sprayed trees 8 weeks after spraying. It became evident at this time, however, that, although the flowers had not dropped, the ovaries were pale yellow in color instead of the usual deep green, and relatively few were developing into fruit. Furthermore, the ovaries failed to enlarge, whereas those on the unsprayed trees were two to three

times as large as at the beginning of the experiment. In late May, with the coming of summer weather, nearly all the flowers that had been retained on the sprayed portion of the trees dropped, and, as a result, with 2,4-D sprays of 30 p.p.m. or more, there appeared to be even fewer young fruits remaining on the sprayed portion of a tree than on the unsprayed. It was also evident from these

er, after application. It seems unlikely that a second spraying during that time would have been any more effective in inducing fruit development than was the first. Although 2,4-D can apparently delay the formation of abscission layers in the flower parts, the effect, in these trials at least, was not such as to stimulate enlargement of the ovary. A different or additional growth factor is probably neces-

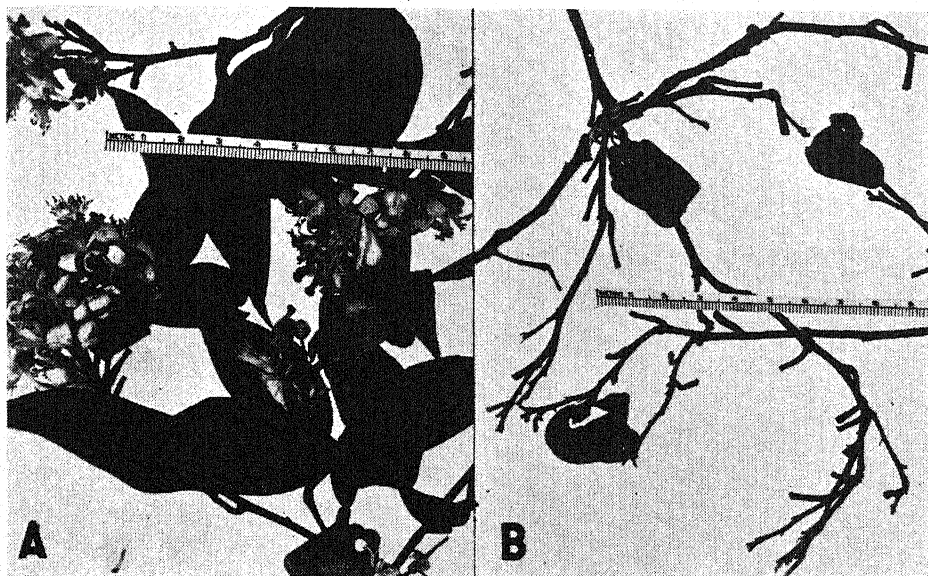


FIG. 3.—Twigs of Valencia orange after 58 hours in ethylene chamber at high humidity: *A*, from portion of tree sprayed 10 days earlier with 60 p.p.m. of 2,4-D; *B*, from unsprayed portion of same tree.

trials that the blossom-retaining effect of 2,4-D was not transmitted from the sprayed portion of the tree to the unsprayed area.

These results were duplicated at the Glendora plot, where concentrations of 15, 30, 60, 120, 240, and 480 p.p.m. of 2,4-D were tested. For each concentration alternate quadrants were sprayed on the north halves of two trees.

It was found that at all concentrations the 2,4-D sprays effectively decreased the usual abscission of ovaries and flower parts for 8–12 weeks, or long-

sary for the enlargement of the ovary. This might be found in pollen extracts or in the stilbene compound recently reported as being very effective in inducing fruit growth of tomato (11).

JUNE DROP.—Studies of the effectiveness of 2,4-D sprays in decreasing June drop of young orange fruits were begun on May 31, 1946. Aqueous sprays containing 25, 75, and 225 p.p.m. of 2,4-D were applied on trees of both Washington Navel and Valencia oranges at Riverside. The Navel plot consisted of six randomized blocks in which east or west halves

of the trees were sprayed, the sides alternating from block to block. The Valencia plot consisted of a Latin-square arrangement of trees, which were sprayed on alternate east or west halves, according to the row. Immediately after spraying, all the leaves and young fruit were removed from underneath the trees, and a heavy circular wire hoop inclosing an area of 10 square feet was placed under the sprayed half of each tree. Subsequent fruit-drop was determined by counting only the fruit within the hoop.

readily with transmitted light. This response was not observed in the leaves of the Navel orange but was noted in leaves on grapefruit trees sprayed with this concentration of 2,4-D (10).

On both Valencia and Navel orange trees, the 75- and 225-p.p.m. sprays decreased the amount of maturing fruit and induced morphological changes in the fruit still remaining on the trees. The 225-p.p.m. treatment on the Valencia trees caused a curious pebbling of the peel of the green fruit (fig. 4). Prelimi-

TABLE 1

EFFECT OF AQUEOUS SPRAYS OF 2,4-D ON JUNE DROP OF YOUNG WASHINGTON NAVEL AND VALENCIA ORANGES. SPRAY APPLIED ON MAY 31, 1946
FIRST FRUIT COUNT, JUNE 13; SECOND COUNT, JULY 12, 1946*

CONCENTRATION OF 2,4-D (P.P.M.)	AVERAGE NO. OF FRUITS DROPPED PER TREE					
	Navels			Valencias		
	First count	Second count	Total	First count	Second count	Total
0 (unsprayed control).....	52	83	135	134	105	239
25.....	61	106	167	68	177	245
75.....	33	149	182	93	149	242
225.....	19	124	143	49	158	207

* In area of 10 square feet under each tree.

In both plots the 2,4-D delayed abscission of the young fruit for 6-8 weeks, but after that time the fruit dropped to the same extent as though the trees had not been sprayed (table 1). In addition, there was severe curling of all foliage expanding at the time of the spray application. Actually, however, as the treatment was made between flushes of leaf growth, little response of this type was observed. The mature leaves of the Valencia orange trees sprayed with 225 p.p.m. of 2,4-D developed chlorotic areas. These were usually found in the tissue between the lateral veins and could be seen most

nary microscopic observations of free-hand sections indicate that this is the result of both outward and inward elongation of the oil glands of the peel. These glands in normal fruits are usually ellipsoidal and imbedded in the peel and do not protrude above the surface of the rind as in the sprayed fruits (6). The rind was also several times thicker on the sprayed fruits than on the unsprayed.

On the same Valencia trees, some fruits became cylindrical in shape and developed a small navel complete with juice vesicles (fig. 4). The spherically shaped fruits on either treated or un-

treated trees showed no comparable navel structure but only the presence of undeveloped vascular strands in the stylar portion. Navel development, cylindrically shaped fruit, and pebbled and thickened peel were likewise found in grapefruit in response to an aqueous spray containing 225 p.p.m. of 2,4-D, applied on June 3, 1946, to trees bearing young fruit (10).

Young Navel fruits sprayed with 225 p.p.m. of 2,4-D showed somewhat less tendency toward pebbled peel than the Valencia fruits but developed a thicker rind than did unsprayed fruit. The sprayed fruit also either developed ex-

cessively large, protruding navels or grew into cylindrically rather than spherically shaped fruit (fig. 5), suggestive of that produced on trees affected with "stubborn" or "acorn" virus disease (3, 5). A few of the unsprayed fruits developed protruding navels, but nearly all the sprayed ones had this characteristic. In addition, all the sprayed fruits examined had seeds or seedlike structures (fig. 6), whereas the unsprayed fruits were seedless, as is usual with this variety of orange. The seeds varied in length from less than a millimeter to over a centimeter, the largest being in fruits on trees sprayed with 75 or 225 p.p.m. of 2,4-D.

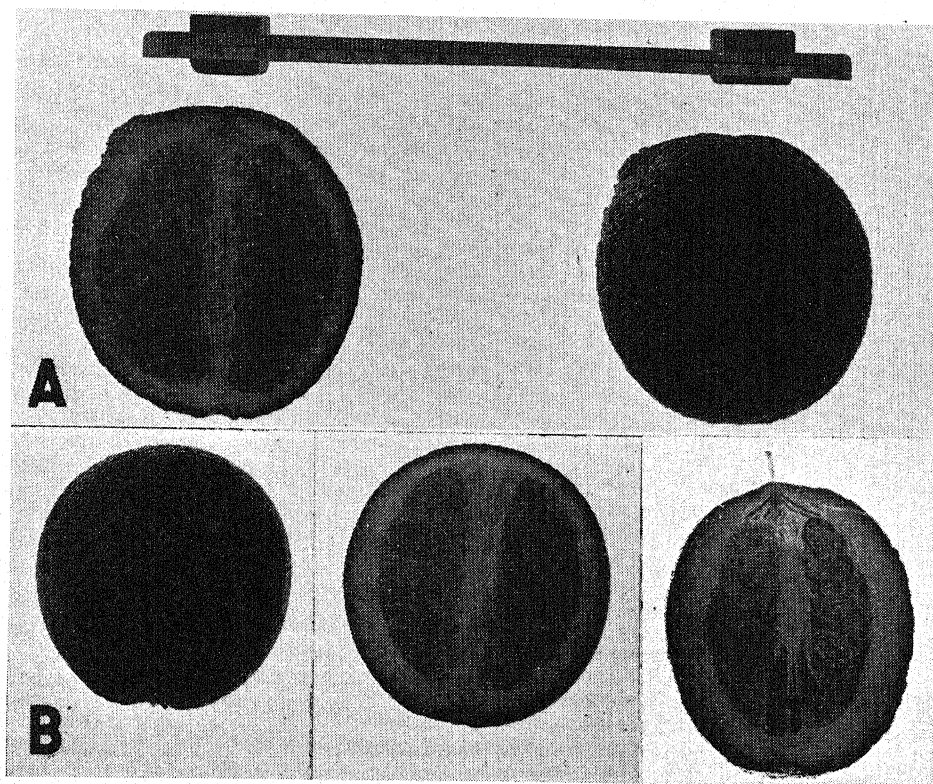


FIG. 4.—A, pebbled appearance of rind of fruit of Valencia orange tree sprayed with 75 p.p.m. of 2,4-D on May 31, 1946. (Photographed October 14, 1946.) Note navel structure and rind proliferation where fruit touched ground. B (left) spherical (normal) shape of fruit from unsprayed control tree; (right) cylindrical shape of fruit from tree sprayed with 225 p.p.m. of 2,4-D. (Photographed November 1, 1946.) Note persistent style and navel structure.

Dissection of a hundred of the largest seeds showed that most of them had embryos, of which only about 15% were over 3 mm. in length. Cultures are being attempted with the largest embryos. Seed counts of seventeen fruits from the trees sprayed with 225 p.p.m. of 2,4-D indicated an average of 53.9 seeds per

On January 9, 1947, the fruits on the sprayed and unsprayed Navel trees were harvested and counted. Fruit size was determined by counting the fruit in each full field box. It was found that the 25-p.p.m. spray had increased fruit size, compared with that of unsprayed fruit and that still greater size increases had

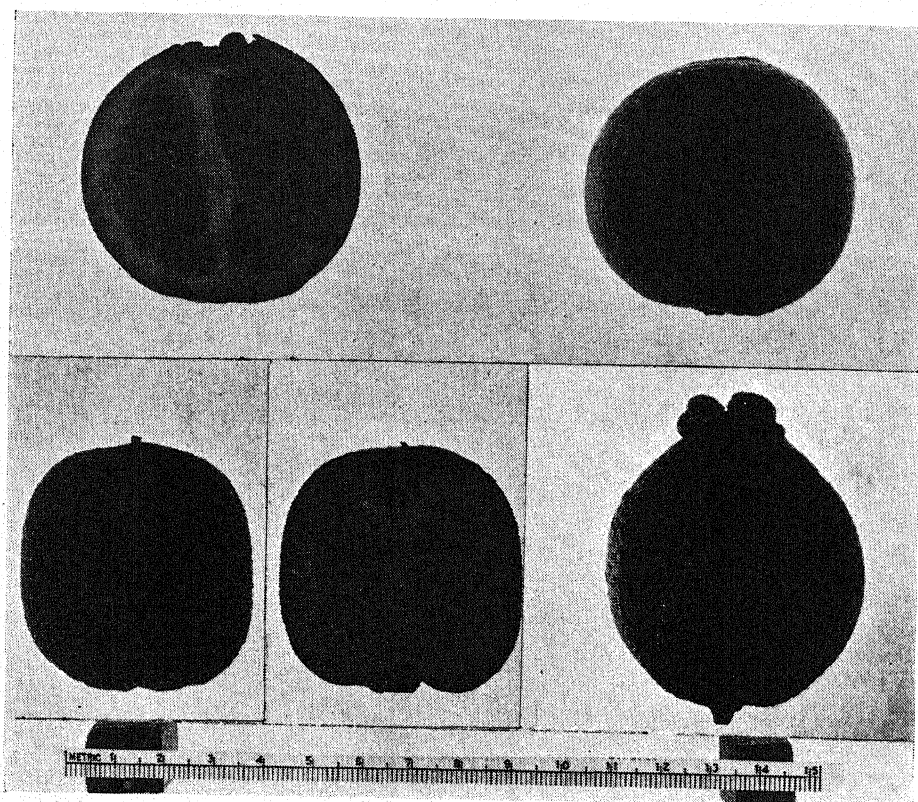


FIG. 5.—Washington Navel oranges. *Above*, normal fruit from unsprayed portion of tree. *Below (left)*, cylindrical shape of some fruit and *(right)* excessive navel growth of other fruit from portion of same tree, to which aqueous spray of 225 p.p.m. of 2,4-D had been applied.

fruit, with a standard error of ± 7.1 . The different responses of the young Navel fruits to high-concentration sprays were probably the result of spraying fruit in slightly different growth stages. As none of these changes in fruit morphology was anticipated, individual fruits were not tagged at the time of spraying.

been obtained in fruit sprayed with 75 or 225 p.p.m. of 2,4-D (table 2). These last two concentrations also resulted in a decreased fruit yield. It is possible, however, that the increases in fruit size were a direct response to the 2,4-D itself and not an indirect response to the fruit-thinning effect of reduced yield, since

hand thinning of Navel oranges results in diameter increases of not more than 8% (8), compared with increases of as much as 25% obtained with the sprays. These data suggest a possible use of 2,4-D sprays for increasing fruit size.

At the time of obtaining the above yield records, composite samples of a

produced larger fruits than the untreated. They also showed that changes in fruit structure accompanied size increases. On the basis of fruit weight, there was an increase in percentage of rind and rag, and a decrease in juice, with increasing concentrations of 2,4-D (table 3). Chemical analyses of the juice

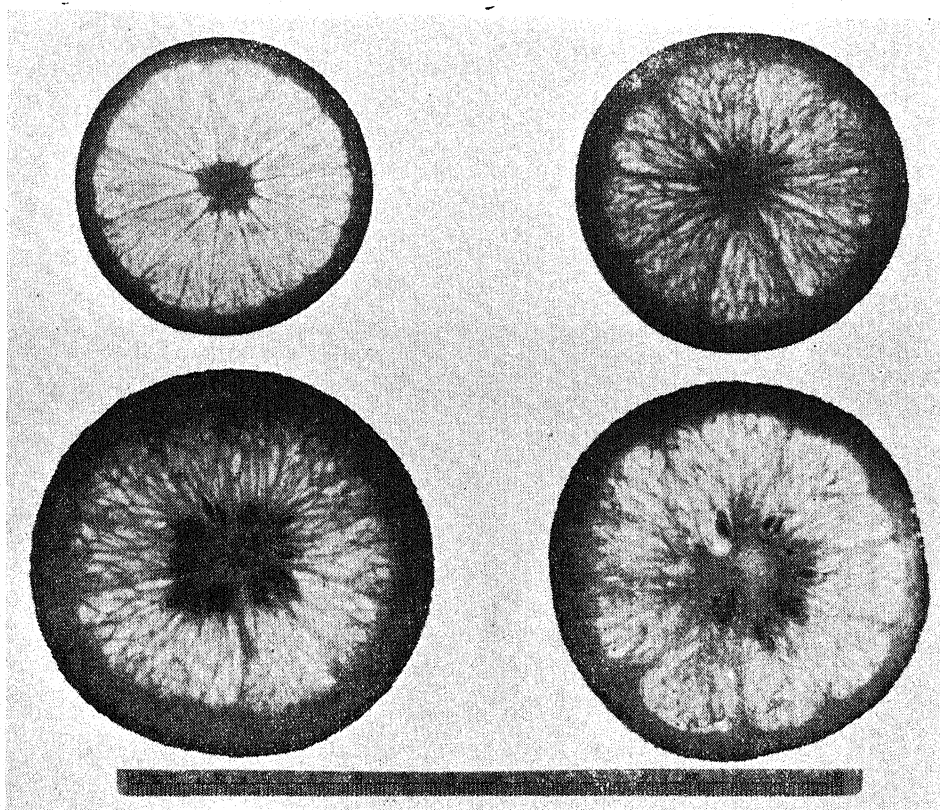


FIG. 6.—Washington Navel oranges showing seedlike structures in fruits from trees sprayed on May 31, 1946, with the following amounts of 2,4-D in water: (*upper right*) 25 p.p.m., (*lower left*) 75 p.p.m., (*lower right*) 225 p.p.m. Fruit at upper left is an unsprayed control. (Photographed November 1, 1946.)

hundred and twenty fruits (twenty from the treated half of each tree) were taken for quality studies. The fruit samples were selected to be representative of the treatments; they were not random samples. Physical measurements confirmed the results of field-box counts and indicated that the treated halves of the trees

indicated a decrease in total acids as a result of the sprays (table 4). Since large fruit has been found to contain a lower concentration of soluble solids than small fruit (1), the slight decreases in soluble solids observed in the large fruit from the treated halves of the trees are not considered significant. The very

slight decreases in ascorbic acid are likewise not considered significant.

The effectiveness of 2,4-D sprays in decreasing June drop of oranges appears to be in essentially the same category as its effectiveness in increasing fruit-set. In both cases the drop was delayed, only to be followed by the same amount of drop as though the spray had not been applied. In fact, at concentrations of 2,4-D above 25 p.p.m., the sprays in-

and in one plot at Camarillo, Ventura County.

Severe preharvest drop is usually an annual occurrence in the Rivera district, apparently as a result of environmental conditions there. The first plot established in this area consisted of five treatments in six replicated, randomized blocks, one tree in each block being used per treatment. In three of the blocks the east half of the tree was sprayed; in the other blocks the west half was treated. Concentrations of 2,4-D used were: 0, 5, 10, 20, and 40 p.p.m. The second plot consisted of treatments of 0, 5, and 20 p.p.m. of 2,4-D. In this plot, as in the June-drop studies, east or west halves of trees were sprayed, the sides alternating from block to block. There were four randomized trees per treatment.

In both plots, immediately after spraying, all fruit on the ground was removed, and subsequent periodic counts were made of the fruit dropped. In addition, in the first plot, fruit remaining on the tree at the end of the experiment was determined for both treated and untreated halves, so that fruit-drop here is expressed as a percentage of the total number of fruits on the halves of the tree at the beginning of the experiment. This eliminates the effect of tree size on the drop counts.

There was a significant reduction in fruit-drop in both Rivera plots as a result of the 2,4-D sprays. In the first plot this varied from 39.6 to 55.7% for the 5-p.p.m. and 40-p.p.m. sprays, respectively (table 5). The decreases in fruit-drop with sprays of 5-20 p.p.m. of 2,4-D were significant at 5%; the decrease with the 40-p.p.m. spray was significant at 1%. In the second plot, where effects of tree size could not be eliminated, the 5-p.p.m. spray failed to decrease fruit-drop significantly; the 20-p.p.m. spray, however,

TABLE 2
EFFECT OF AQUEOUS SPRAYS OF 2,4-D ON YIELD AND SIZE OF WASHINGTON NAVEL ORANGE FRUITS. SPRAYS APPLIED TO ONE-HALF OF EACH TREE ON MAY 31, 1946; FRUIT HARVESTED ON JANUARY 9, 1947

CONCENTRATION OF 2,4-D (P.P.M.)	YIELD (AVERAGE NO. OF FRUITS FROM HALVES OF SIX TREES)		SIZE (AVERAGE NO. OF FRUITS PER FIELD BOX, FROM HALVES OF SIX TREES)	
	Sprayed	Un- sprayed	Sprayed	Un- sprayed
0 (unsprayed control).....		300		163
25.....	327	256	147	182
75.....	186	272	111	164
225.....	73	289	100	137

duced greater delayed drop, so that production was actually decreased. The increase in size of the remaining fruit suggests possible use of these sprays to increase fruit size; however, at the expense of commercial quality. Although satisfactory results for the control of June drop were not obtained, there are indications from the data that the problem may be solved by further studies in this direction.

PREHARVEST DROP.—The effect of 2,4-D on preharvest drop of Valencia oranges was studied in two field plots at Rivera, Los Angeles County, California,

effected a decrease of 49.3%, which was significant at the 5% level (table 5).

The Camarillo plot was established on September 5, 1946, late in the harvest season for Valencia oranges. There had been a heavy fruit-drop during the preceding 2 weeks, and an estimated two hundred to three hundred fruits per tree had already dropped before the treatment was applied. This plot consisted of forty trees, twenty trees in each of two adjacent rows. Ten of the trees in each row were sprayed with 25 p.p.m. of 2,4-D, and the others were left un-

sprayed for comparison. The entire tree was treated. In each row the sprayed and unsprayed trees alternated, so that, in comparing the trees in the two adjacent rows, one always found a treated tree opposite an untreated one. Immediately after spraying, all fruits were removed from the ground under the trees.

On October 9 the fruit on the ground was counted (fig. 7) and that remaining on the trees was harvested and counted. On the basis of the total amount of fruit on the trees at the beginning of the experiment, there was an average drop of

TABLE 3

EFFECT OF AQUEOUS SPRAYS OF 2,4-D ON PHYSICAL-QUALITY FACTORS OF WASHINGTON NAVEL ORANGE FRUITS FROM HALVES OF TREES SPRAYED ON MAY 31, 1946;
FRUITS HARVESTED ON JANUARY 9, 1947*

CONCENTRATION OF 2,4-D (P.P.M.)	WHOLE FRUIT			RIND† (WEIGHT)		RAG‡ (WEIGHT)		JUICE (WEIGHT)	
	Volume (ml.)	Diameter (cm.)	Weight (gm.)	Gm.	% of whole fruit	Gm.	% of whole fruit	Gm.	% of whole fruit
0 (unsprayed control).....	143.5	6.45	130.7	61.8	47.3	1.39	1.1	67.5	51.6
25.....	210.6	7.49	178.0	89.9	50.5	5.54	3.1	82.6	46.4
75.....	261.2	7.63	227.4	122.5	53.9	8.40	3.7	96.5	42.4
225.....	293.9	8.05	256.9	137.5	53.6	13.80	5.4	105.6	41.0

* Composite samples of 120 fruits each.

† Flavedo, albedo, and any additional attached tissues remaining after reaming fruit on electric juice extractor.

‡ Tissues not passing through vibrating sieve on electric juice extractor.

TABLE 4

EFFECT OF AQUEOUS SPRAYS OF 2,4-D ON CHEMICAL-QUALITY FACTORS OF WASHINGTON NAVEL ORANGE FRUITS FROM HALVES OF TREES SPRAYED ON
MAY 31, 1946; FRUIT HARVESTED ON JANUARY 9, 1947*

Concentration of 2,4-D	pH	Total acid, as citric (%)	Soluble solids (%)	Ratio of soluble solids to total acid	Ascorbic acid (mg./100 ml. juice)
0 (unsprayed con- trol).....	3.29	1.37	12.72	9.28:1	61.53
25.....	3.29	1.21	12.58	10.40:1	60.06
75.....	3.39	1.15	12.51	10.53:1	57.13
225.....	3.43	1.14	12.58	11.03:1	58.60

* Composite samples of 120 fruits each.

TABLE 5
PREHARVEST FRUIT-DROP IN TWO PLOTS OF VALENCIA ORANGE TREES AT RIVERA, CALIFORNIA
ALTERNATE HALVES OF TREES SPRAYED WITH AQUEOUS SOLUTIONS OF
2,4-D ON MAY 17, 1946

CONCENTRATION OF 2,4-D (P.P.M.)	PLOT 1						PLOT 2	
	Percentage of fruits dropped May 17 to August 10, 1946 (average from halves of six trees)*						No. of fruits dropped, May 17 to July 8, 1946 (average from halves of four trees)	
	Computed from trees in randomized blocks			Computed from paired compar- ison of sprayed and unsprayed halves of same tree				
	Un- sprayed	Sprayed	Decrease due to spraying	Un- sprayed	Sprayed	Decrease due to spraying		
	Un- sprayed	Sprayed	Decrease due to spraying	Un- sprayed	Sprayed	Decrease due to spraying	Un- sprayed	Sprayed
0 (unsprayed con- trol).....	10.6†						24.5	
5.....		6.4	39.6	9.27	6.37	31.3‡	29.5	28.5
10.....		7.5	29.3	10.79	7.53	30.2‡		
20.....		6.0	43.4	10.40	5.95	42.8‡	22.3	11.3§
40.....		4.7	55.7	12.27	7.61	38.0		

* Percentage of total fruit on trees at time of spraying.

† Least significant difference at 5% level, 2.49; at 1% level, 3.36.

‡ Significant at 5% level.

§ Significant at 5% level by *t*-test, in comparison with average fruit-drop (25.6) based on the sixteen unsprayed tree halves.

|| Significant at 1% level.

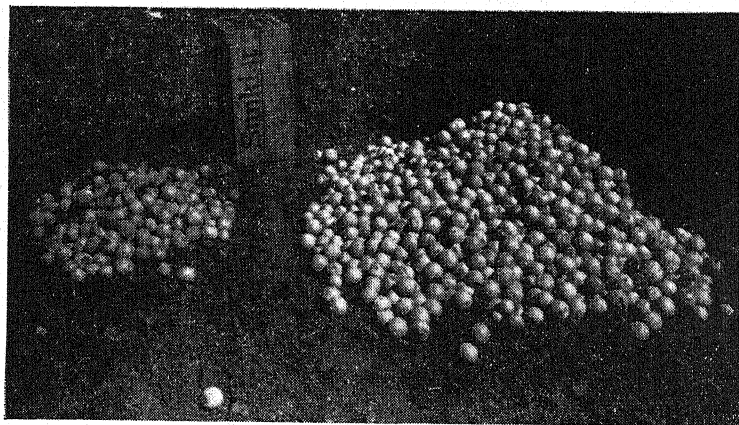


FIG. 7.—Valencia oranges dropped between September 5 and October 9 at Camarillo plot: (left) from twenty trees sprayed with 25 p.p.m. of 2,4-D and (right) from twenty unsprayed trees.

11.05% from the unsprayed trees, compared with 2.47% from the sprayed ones. This difference of 78% is significant at the 1% level. The number of fruits dropped from the twenty unsprayed trees was 2,861, compared with 599 from the sprayed trees. In addition, most of the fruit dropped from the unsprayed trees was firm and of a better quality than that dropped from the sprayed ones. The spray treatment thus not only decreased drop but also resulted in a drop of only the poor-quality fruit.

Fruit-quality analyses of representative composite samples of one hundred fruits each from twenty unsprayed and from twenty sprayed trees indicated no significant differences in soluble solids, total acid, pH, or ascorbic acid.

With the preharvest sprays, as with the fruit-set and June-drop sprays, it was observed that a curling of the expanding young leaves occurred at concentrations of 2,4-D above 5 p.p.m. At 5 p.p.m. leaf curling was barely perceptible. When the sprays were applied between leaf-growth flushes, little or no curling of leaves was apparent.

In the plots at both Rivera and Camarillo the treatments were applied after fruit-drop had started. GARDNER (5), in Florida, reported that, in the use of sprays of naphthaleneacetic acid and naphthaleneacetamide, the more in advance of the drop the application was made, the less was the drop. When applied 3-4 months before the drop, they induced the greatest decrease; their application when fruit was dropping caused little or no decrease in fruit-drop. In contrast, the 2,4-D sprays decreased fruit-drop as much as 78% when applied even after the drop had been occurring for 2 weeks. At present, the main difficulty in the use of 2,4-D sprays is their curling effect on new foliage. It is not known to

what extent, if any, curling may impair the efficiency of the leaves or be a serious objection. The spray would be applied only once a year for preharvest fruit-drop, whereas there are at least three leaf-growth flushes a year. For this reason, only one set of new leaves would be affected, and if the spray were applied between growth flushes even this amount of leaf injury might be avoided.

Summary

1. Some effects of aqueous sprays of 2,4-dichlorophenoxyacetic acid (2,4-D) on fruit-drop and morphology of oranges are reported.

2. When applied to Valencia orange trees at full bloom, these sprays delayed blossom-drop 8-10 weeks, or more, but did not increase fruit-set. Applications in May likewise delayed the June drop of immature Washington Navel orange fruits 6-8 weeks but did not increase yield.

3. Young Navel fruits sprayed at this time with concentrations of 25-225 p.p.m. of 2,4-D developed seeds, or seed-like structures, in contrast to unsprayed fruits, which were seedless. Some fruit on the trees sprayed with concentrations of 225 p.p.m. of 2,4-D developed a thick rind and grew excessively large, protruding navels; other fruits became cylindrical in shape.

4. Fruit on Valencia orange trees treated in May with a concentration of 225 p.p.m. of 2,4-D developed a thick, pebbly rind. Other fruit on these trees became cylindrical in shape and developed small navels.

5. Applied to Valencia orange trees bearing mature fruit, the sprays decreased preharvest fruit-drop as much as 78%, even when applied 2 weeks after severe drop had begun.

6. Until additional data are available

from studies now in progress, these sprays are not recommended for commercial use.

The authors appreciate the generous and willing co-operation of the owners of the groves used in this work. They thank the Thompson Horticultural Chemicals

Corporation of Los Angeles for furnishing the 2,4-dichlorophenoxyacetic acid. The assistance of Mr. LOUIS BRANNAMAN and Mr. HOWARD EVANS greatly facilitated these studies.

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EFFECTS OF 2,4-DICHLOROPHENOXYACETIC ACID ON GAS EXCHANGE OF WHEAT AND MUSTARD SEEDLINGS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 592

D. L. TAYLOR¹

Introduction

Information has been contributed by many investigators concerning the nature of responses of various tissues and plants to variations of treatment with the substituted phenoxyacetic acids, especially 2,4-dichlorophenoxyacetic acid (2,4-D). The present wide use and indicated possibilities for even broader uses

of these chemicals make it most desirable to obtain more information as to the way, or ways, in which they influence metabolism. Several investigators have reported on the hydrolytic and mobilization responses involving reserves in a number of dicotyledonous species after treatment with chemical growth-regulators (14, 15, 21, 22, 26). Often the amount of mobilized carbohydrate has been observed to decrease before the end

¹ Deceased December 6, 1947.

of the tests or prior to the death of treated plants.

BROWN (7), who investigated water relations and the distribution of solid matter in kidney beans, used a volumetric method with an 18-hour test period and found distinctly increased carbon dioxide (CO_2) evolution by beans 5-7 inches tall and by mature morning-glory plants sprayed with 1000 p.p.m. of 2,4-D. By means of manometric technique SMITH *et al.* (21) recently observed increased rates of oxygen (O_2) uptake by segments of underground stems of bindweed field-sprayed with 2,4-D. It has been suggested that starvation may have developed as a part of the complex of responses, resulting in the eventual death of treated plants. An interpretation has been made that increased utilization of reserves resulting from stimulated respiratory activity may have contributed toward development of starvation.

Since November, 1946, many tests have been conducted on mustard, radish, wheat, barley, and rice seedlings in a study of effects of 2,4-D on their metabolism of energy release. This paper presents results on the intensity and duration of treatment with 2,4-D as correlated with the alteration of gas-exchange rates of mustard and wheat seedlings at different stages of seedling development.

Material and methods

Certified seed of Henry variety of spring wheat (*Triticum vulgare*) and white mustard (*Brassica alba*) were used. The 2,4-D, recrystallized twice from material of a technical grade, was a small crystalline, white powder, m.-p. 134° - 136° C. The rates of O_2 uptake and CO_2 evolution of samples of seedlings which had been variously treated with 2,4-D were measured with standard Warburg manometric equipment, using details of

the technique for the direct (two-vessel) method (27).

In preparation for a test, sound seeds were stirred 4 minutes in a saturated, filtered solution of calcium hypochlorite. After subsequent thorough stirring in six changes of water, the seeds were spread on two layers of filter paper in moist-chambers 8 inches in diameter which contained 10-20 ml. of free water. Germination progressed in the dark in an incubator at 28° C. for wheat and at 25° C. for mustard until the seedlings were of an age desired for treatment, after which large numbers of uniform seedlings were selected, divided into lots, and each lot placed in the desired treatment solution. The period of germination was always considered to begin at the time of treatment to disinfect seeds.

All manometric measurements were made in the dark at $28 \pm 0.1^\circ$ C. in solutions buffered by $M/100$ monopotassium acid phosphate. In all experiments the manometers were moved 110-120 strokes per minute. It was established that the activity of standard seedling samples in air and in nitrogen did not vary when manometers were moved at more than 90 strokes per minute and that the activity of untreated seedlings increased uniformly in air during test periods lasting 3 hours. The O_2 content of the air in the vessels or the diffusion of gas in the substrate solution (2.5 ml.) apparently did not limit aerobic activity under these conditions.

Manometers were read at intervals of 10-15 minutes during test periods of 45-100 minutes, depending upon the intensity of activity and the size of sample tested. The rates of O_2 uptake and CO_2 evolution and the size of seedlings varied for different kinds and ages. These qualities were factors considered in determin-

ing the numbers of seedlings of different kinds or ages used in manometric tests.

Eight to twenty-five selected seedlings were used in each replicate. The number used per replicate was smaller as the seedlings became older and was larger for mustard than for wheat seedlings of the same age. The same numbers of seedlings of a species were used in the replicates of all treatments in different tests of seedlings of any specific age.

Manometric data were converted to volume values, and the rates of O_2 uptake and CO_2 evolution were calculated (27) on a seedling basis. In previous studies on wheat (19, 24) and on other cereals (4, 12, 24), embryonic activity has been found to account for 80-90%, or more, of the total seedling utilization of O_2 or of CO_2 evolved. Therefore, embryos (minus scutella) were removed from the wheat seedlings tested, dried to constant weight, and the activities calculated on the basis of 1 mg. dry weight of such embryonic tissue.

Mustard seedlings proved to be much less satisfactory for study than wheat. Rates of O_2 uptake and CO_2 evolution were relatively low, and, though larger samples— 20 ± 5 seedlings—were tested, the variations in results (up to 10%) were often greater than those for wheat (8% or less). Subdivision of mustard seedlings into relatively active and inactive parts was not possible as it was for wheat. Values for untreated mustard were obtained which were relatively very low when data for some of the initial tests were calculated on the basis of unit of dry weight of seedling tissue (see data for seedlings 70 hours old, table 3). Since no consistent advantage resulted from such calculations, weight data were not obtained in several of the tests, and the results are presented on the basis of seedling samples only.

Several advantages are gained from manometric techniques, even though the number and the size of seedlings (the amount of tissue) and the length of a test period without resetting of the apparatus tend to be somewhat limited. Accurate data could be obtained from numerous, comparatively brief tests (11) in which relatively small samples of intact seedlings, or seedling tissues, could be subjected to a variety of conditions of environment and treatment with 2,4-D according to techniques which have been used, fully described, evaluated, and accepted by numerous individuals.

Some advantages in using seedlings, or tissues thereof, were the ease of obtaining ample, uniform test material in minimum time; the normal occurrence of very high activity in such material; and the ease of accomplishing significant variations of treatment. Greater reproducibility of samples of tissue used in a test and between results of different tests and replicates of a treatment was found to be possible if the seedlings were selected for treatment when their early development could be determined to be normal. It should be kept in mind that previous studies have shown that a succession of rather marked qualitative and quantitative changes in activity normally occurs during the development of many seedlings or their parts (1, 6, 10, 12).

Experimentation

In a number of initial tests information was obtained concerning the degree of apparent response of the metabolism of energy release in relation to duration of treatment. Some details of technique in later tests were determined in part according to results of these tests. The rate of O_2 uptake was measured for representative, fifteen-seedling samples of wheat

and mustard during the twenty-fourth hour after seeds were disinfected. When seedlings were 24 hours old, buffered 2,4-D solution was tipped from a side-arm into the $M/100$ phosphate buffer in the seedling chamber of the vessel and thoroughly mixed so that the 2,4-D concentrations which resulted were 0, 1, 2.5, or 5 p.p.m. During the first and second hours after addition of 2,4-D the O_2 uptake by the samples was measured (table 1).

Earlier studies (10, 12) have shown that marked changes in the rate of respiration parallel to rapid growth oc-

curred in seedlings during a similar period. It would seem possible that the rate of activity of the samples might reflect some such change even during relatively short test periods. The progressive development of the effect of the treatment with 2,4-D on growth (size of sample) might be a factor to be considered in evaluation of the metabolic responses of variously treated samples of seedlings. Treatment of data for gas exchange on the basis of weight of embryonic tissue might facilitate evaluation or elimination of the effect of the acid on growth of the sample. Adjustment for unavoidable

TABLE 1
PROGRESSIVE DEVELOPMENT OF EFFECT OF 2,4-D ON RATE OF O_2 UPTAKE BY WHEAT
OR MUSTARD SEEDLINGS 25 HOURS OLD. ACTIVITIES SHOWN
AS CU. MM./HR./10 SEEDLINGS

TIME OF MEASUREMENT	TREATMENT (P.P.M.)							
	Wheat				Mustard			
	0	1	2.5	5	0	1	2.5	5
Measured rate*								
$T-1$	183	174	174	178	35.0	36.0	42.0	53.0
$T+1$	192	168	180	172	41.0	30.0	34.0	59.0
$T+2$	196	162	174	174	37.5	24.0	28.0	50.0
Change in rate measured†								
$T+1$	+5	-3	+3	-3	+17	-17	-19	+11
$T+2$	+7	-5	0	-2	+7	-33	-33	-6
Difference between measured and expected rates‡								
$T+1$		-8	-2	-8		-19	-31	-5
$T+2$		-13	-7	-9		-38	-38	-12

* Base rate for each sample was determined during hour ($T-1$) immediately prior to time (T) of addition of 2,4-D to substrate. Measurements of activities were made during first ($T+1$) and second ($T+2$) hours after treatment.

† Changes are expressed in relation to base rate ($T-1$) of each sample valued as 100.

‡ Changes in rate for untreated seedlings, measured 1 and 2 hours after T , were applied as factors to base rates ($T-1$) measured for treated samples to obtain values for latter samples which would be expected in the absence of 2,4-D during 1 and 2 hours after T . Differences between measured and expected rates for each sample are shown in relation to 100 assigned to expected rate for each sample at 1 or at 2 hours after T .

differences in volume size of samples might also be possible.

In the protocols (fig. 1) for control wheat (*W-C*) and mustard (*M-C*), lines 1 and 2 converge toward line *B* at the top. This indicates a progressive increase in rate of O_2 uptake during the test. Evidence of growth was not visible, and other growth data were not obtained. In other similar tests it was not possible to measure a significant increase in the weight of embryonic tissue of samples during such test periods. However, this

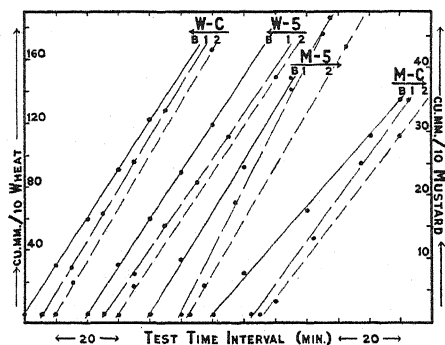


FIG. 1.—Protocols of tests of O_2 uptake by wheat (*W*) and mustard (*M*) seedlings: (*B*) untreated during twenty-fourth hour of development, (*C*) untreated and (*5*) treated with 5 p.p.m. of 2,4-D during twenty-fifth and twenty-sixth hours (1 and 2, respectively) of development. Treatments applied to seedlings 24 hours old.

relation for controls most likely is an expression of small, possibly unmeasurable growth which accompanied or resulted in the increase in O_2 uptake of the samples. Change in rate of O_2 uptake per unit weight of tissue possibly, but not necessarily, was involved.

Base rates of O_2 uptake were different for different samples (table 1, measured rates at $T-1$). It seems probable that the volumes of embryonic tissue would have varied somewhat among samples and that initial rate was proportional to sample volume. During the 2 hours of treatment, rates of O_2 uptake of treated

samples did not increase as much as did those of untreated samples (table 1, change in rate measured). Protocol O_2 uptake lines (1 and 2) for seedlings in 5 p.p.m. of 2,4-D converged less toward the base rate lines (*B*) than they did for respective control samples during the first and second hours after treatment (fig. 1). This may indicate an effect of 2,4-D on growth (weight of sample), an effect on rate of O_2 uptake per unit weight of tissue, or a complex involving both effects.

A smoother relation between rate of O_2 uptake and concentration or duration of treatment with 2,4-D resulted when the data for measured rates of treated samples were somewhat adjusted to account for the probable variation in initial volume of the samples (table 1, difference between measured and expected rates). Such calculation shows that the rate of O_2 uptake of wheat and mustard seedlings had been reduced 7–38% during 2 hours following application of 1 p.p.m., or more, of 2,4-D. The response was proportional but not parallel to duration of treatment and was irregular in relation to concentration of 2,4-D.

In all instances after 2 or 18 hours of pretreatment with 2.5–10 p.p.m. of 2,4-D (table 2), the rates of O_2 uptake and of CO_2 evolution by intact wheat seedlings 30 or 48 hours old were reduced compared with controls. The decreases in rates of activity were somewhat proportional to concentration and to duration of treatment with 2,4-D. Slightly greater decrease in rate of O_2 uptake than in rate of CO_2 evolution occurred in seedlings 48 hours old, so that respiratory quotients (*RQ*) of the treated samples were slightly higher than those of controls.

During the test period of 18 hours the mean dry weight of untreated samples

increased 155%. Increase in dry weight of embryo of treated samples was 18–20% less than in controls. When evaluated on the basis of unit dry weight of embryo, decreases in activities resulting from treatment were always appreciably less and were more clearly and uniformly related to factors of concentration and duration of treatment than they were

twenty-five seedlings 74–78 hours old, pretreated for 2–6 hours with 0.25–5 p.p.m. of 2,4-D, were in all cases lower than those for controls (table 3, fig. 2). As in wheat, the amount of reduction of an activity was generally proportional to concentration and duration of treatment with 2,4-D. Differences in initial samples may in part explain the fact that treated

TABLE 2

MEAN RATES OF CO₂ EVOLUTION AND OF O₂ UPTAKE AND *RQ* VALUES FOR WHEAT SEEDLINGS 30 OR 48 HOURS OLD, TREATED FOR 2 OR 18 HOURS WITH 2,4-D. RELATIVE RATES SHOWN FOR DUPLICATE TREATED SAMPLES, 10 SEEDLINGS EACH, IN COMPARISON WITH VALUE OF 100 FOR MEAN RATE OF UNTREATED SAMPLES IN EACH TEST

SEED- LING AGE (HR.)	PRE- TREAT- MENT TIME (HR.)	2,4-D CON- CEN- TRATION (P.P.M.)	RATE OF ACTIVITY				<i>RQ</i>		DRY WEIGHT OF EMBRYO
			CO ₂ (cu. mm./hr.)		O ₂ (cu. mm./hr.)		Per 10 seedlings	Per mg. of embryo	
			Per 10 seedlings	Per mg. of embryo	Per 10 seedlings	Per mg. of embryo			
30	2	0	221.1	15.28	217.1	15.24	1.02	1.04	13.3 ± 1.4 mg.*
		2.5	82	97	85	99	0.99	0.99
		5	89	95	89	93	1.02	1.02
		10	85	91
48	18	0	384.9	11.06	394.2	11.42	0.98	0.97	34.0 ± 1.2 mg.†
		2.5	70	93	69	89	0.98	1.01	82 ± 6†
		5	68	85	66	82	1.01	1.01	78 ± 4†
		10	57	82	54	77	1.02	1.02	71 ± 8†

* Mean, and maximum variation per 14 replicates.

† Mean, and maximum variation per 3 replicates.

when calculated on the basis of seedling samples.

The *RQ* values, very close to 1.0 for untreated seedlings, suggest the functioning of an aerobic, hexose-type metabolism. Although treatments inhibited growth, the slightly higher *RQ* values for treated seedlings 48 hours old might be interpreted to indicate that anaerobic CO₂ evolution either was evoked or was not inhibited as much as aerobic CO₂ evolution.

Rates of CO₂ evolution and O₂ uptake by samples of mustard consisting of

seedlings 78 hours old were heavier than controls.

In comparison with controls in this test, for all treatments the reduction of CO₂ evolution was as great as, and usually greater than, reduction of O₂ uptake. The *RQ* values of treated seedlings were lower than those of controls. The latter were higher than in other tests of mustard (table 6). Aerobic oxidation processes in treated seedlings may have been less nearly complete than in controls, or treatment may have altered the type of substrate utilized in such seedlings which

have relatively low carbohydrate reserves but are relatively high in fat. To the extent that the oxidative processes may be similar and may be similarly affected by treatment, the comparative responses of wheat and mustard seedlings suggest that anaerobic metabolism in mustard is potentially relatively weak. The influence of 2,4-D on such metabolism likely would not be very great.

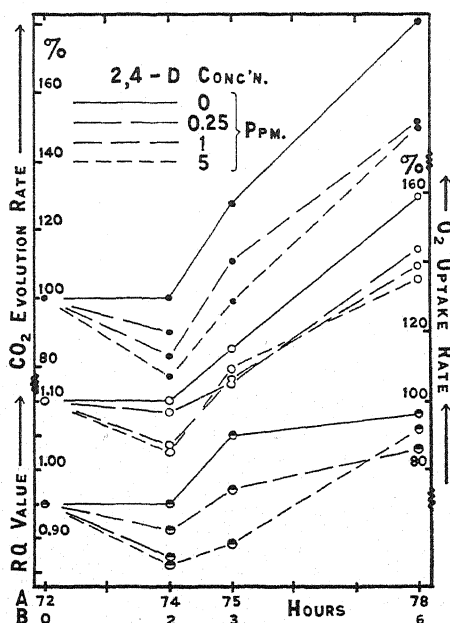


FIG. 2.—Mean relative rates of CO_2 evolution (solid dots) and O_2 uptake (open dots), and RQ values (semisolid dots) measured when duplicate twenty-five-seedling samples of mustard, 70 hours old, were treated with 0–5 p.p.m. of 2,4-D. Age of seedlings shown on line A and duration of treatment on line B.

Compared with controls, changes in the rates of CO_2 evolution and O_2 uptake and in the RQ values for mustard seedlings 26–48 hours old, pretreated for 2–24 hours with 2.5 or 10 p.p.m. of 2,4-D (table 4), were in general similar to those for older seedlings (table 3). Relative to the amount of decrease in rate of O_2 uptake resulting from treatment, the rates

of CO_2 evolution and the RQ values for seedlings 32–48 hours old were reduced less than for seedlings 78 hours old. This could be considered partial evidence that anaerobic metabolism in the younger seedlings was potentially greater in amount or was less sensitive to 2,4-D than in the older seedlings.

When the data were treated to show cumulative change in the relative rates of activities (table 4), the distinct influence of treatment with 2,4-D in limiting the growth of seedlings, as well as in affecting their rates of metabolic activity, became more apparent. CO_2 evolution was only slightly nearer to that of controls than was O_2 uptake for the seedlings up to 32 hours old. The O_2 uptake of seedlings 48 hours old was appreciably more like that of controls than was CO_2 evolution. Altogether these data for mustard seem to give little clear indication of anaerobic metabolism greater or less sensitive to 2,4-D than aerobic metabolism in younger as compared with older seedlings.

On the basis of the tests described, and others, it appeared that 2,4-D caused marked effects which were expressed strongly within 3 hours following application of treatment. A number of tests were conducted in which seedlings of different ages (fig. 3) were pretreated for 3 hours immediately prior to measurement of their rates of gas exchange. Differences in the sizes and relative activities of seedlings of different types and ages made it desirable to use samples of different sizes in various tests. Data are summarized in terms of samples consisting of ten seedlings to facilitate comparison between activities of a type of seedling of any age in different tests. The data for wheat were also analyzed on the basis of unit dry weight of embryo (table 5, fig. 4).

TABLE 3

MEAN RATES OF CO₂ EVOLUTION AND OF O₂ UPTAKE AND RQ VALUES FOR MUSTARD SEEDLINGS 74 TO 78 HOURS OLD, TREATED FOR 2 TO 6 HOURS WITH 2,4-D. ACTIVITIES FOR DUPLICATE SAMPLES EXPRESSED ON BASIS OF 10 SEEDLINGS

SEEDLING AGE (HR.)	PRE-TREATMENT TIME (HR.)	2,4-D CONCENTRATION (P.P.M.)	CO ₂ EVOLUTION		O ₂ UPTAKE		RQ	MEAN DRY WEIGHT (MG.)
			Cu. mm./hr.	%	Cu. mm./hr.	%		
74	2	0	128.2	100	135.3	100	0.95	24.0 ± 1.5†
		0.25	113.1	90	131.3	97	0.87	
		1	106.5	83	117.0	87	0.91	
		5	99.3	77	115.1	85	0.86	
75	3	0	163.5	100	156.0	100	1.05	
		0.25	142.9	87	142.2	91	0.97	
		1	127.5	78	147.4	94	0.97	
		5	127.5	78	143.0	92	0.89	
78	6	0	232.0	100	215.4	100	1.08	24.5‡
		0.25	9.09*	100*	8.49*	100*	1.07*	
		0.25	194.2	84	195.2	91	1.03	26.2‡
		1	7.11*	78*	7.35*	87*	1.03	
		1	192.9	85	188.3	87	1.03*	27.6‡
		5	6.86*	73*	6.88*	81*	1.03*	
		5	192.9	85	182.7	85	1.06	26.6‡
		5	6.86*	73*	6.57*	75*	1.04*	

* Calculated per mg. dry weight of embryo.

† Twelve replicates.

‡ Three replicates.

TABLE 4

EFFECTS OF 2,4-D ON GAS-EXCHANGE RELATIONS OF MUSTARD SEEDLINGS. MEAN VALUES SHOWN FOR ACTIVITIES OF DUPLICATE SAMPLES SELECTED AT 24 HOURS AND THEN TREATED WITH 2,4-D FOR 2-24 HOURS PRIOR TO MEASUREMENT OF ACTIVITIES

SEED- LING AGE (HR.)	TREATMENT		MEASURED RATE OF ACTIVITY		RQ	RELATIVE RATE OF ACTIVITY			
	Time (hr.)	Concen- tration (p.p.m.)	Cu. mm./hr. per 10 seedlings			Per seedling age*		Cumulative change in rate†	
			CO ₂	O ₂		CO ₂	O ₂	CO ₂	O ₂
26	2	0 2.5 10‡	34.8 31.5 29.3	40.7 39.4 37.0	0.86 0.80 0.79	100 91 84	100 97 91	0 - 9 - 16	0 - 3 9
27	3	0 10	44.0 38.7	100 88	+ 8 - 5
32	8	0 2.5 10	51.9 44.6 41.2	59.1 51.5 46.6	0.87 0.87 0.88	100 86 80	100 87 79	+ 49 + 28 + 18	+ 46 + 27 + 15
48	24	0 2.5 10	74.1 64.0 55.6	98.2 90.7 78.7	0.75 0.71 0.71	100 86 75	100 92 80	+ 113 + 84 + 60	+ 142 + 123 + 94

* Activities of untreated seedlings of each age valued as 100.

† Activities of untreated seedlings 26 hours old valued as 100.

‡ Only one determination of O₂ uptake.

Progressive increase in the rates of O_2 uptake and CO_2 evolution per ten control seedlings clearly reflect the increase in size of wheat (table 5) and mustard (table 6) seedlings as they became older. The change in rates per milligram of wheat tissue showed that the fraction of

the seedling having lower rates of activities increased with age. The RQ values for mustard clearly tended to decrease as the seedlings became older, likely indicating a progressive depletion of carbohydrate reserve and a shift toward a noncarbohydrate substrate for metabo-

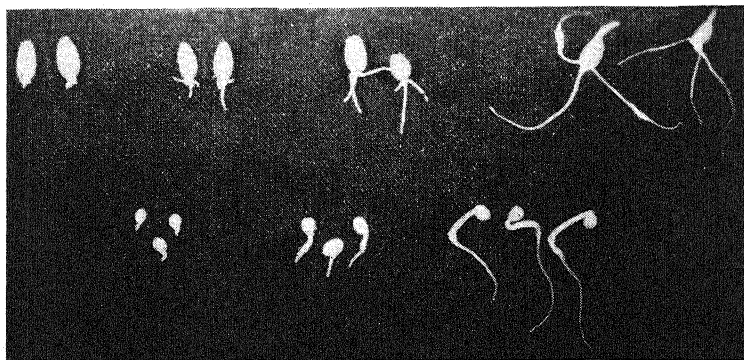


FIG. 3.—Representative seedlings at time of treatment. *Upper (left to right)*, wheat 18, 24, 30, and 50 hours old; *lower (left to right)*, mustard 30, 50, and 70 hours old.

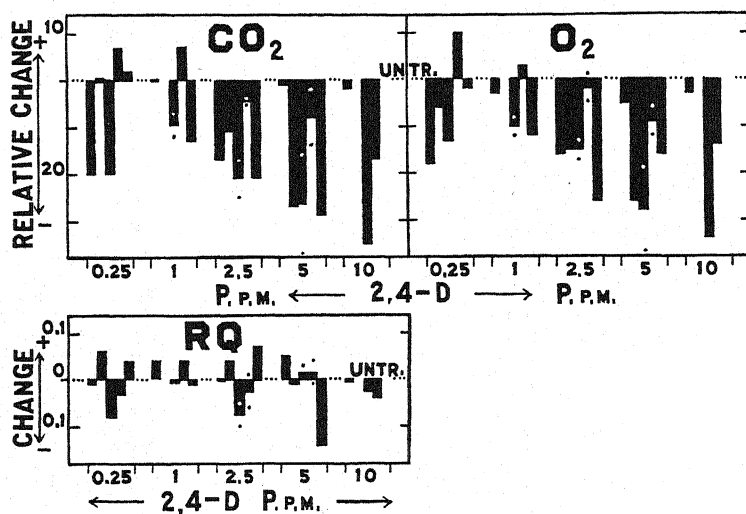


FIG. 4.—Mean relative changes in volume rates of CO_2 evolution and O_2 uptake and changes in RQ values for wheat seedlings 18–60 hours old after 3 hours under treatment with 0.25–10 p.p.m. of 2,4-D. For each 2,4-D concentration five positions are shown, representing (*left to right*) seedlings 18, 24, 30, 50, and 60 hours old. Data calculated on the basis of 1 mg. of dry weight of embryonic tissue. In all tests activities of untreated seedlings valued as 100 (0 on figure scale). Dots appearing in line vertically with a bar indicate maximum variations of means for activities observed in various tests involving age of seedling and treatment concerned. No tests made on seedlings 24 hours old with 1 or 10 p.p.m. or on seedlings 60 hours old with 10 p.p.m.

TABLE 5

MEAN RATES OF CO₂ EVOLUTION AND OF O₂ UPTAKE AND RQ VALUES FOR SAMPLES OF WHEAT SEEDLINGS 18-60 HOURS OLD TREATED FOR 3 HOURS WITH 0.25-10 P.P.M. OF 2,4-D

SEED- LING AGE (HR.)	TEST NO.	2,4-D CONCEN- TRATION (P.P.M.)	RATE OF ACTIVITY OF SEEDLINGS AND OF SEEDLING TISSUE				RQ		
			CO ₂ (cu. mm./hr.)		O ₂ (cu. mm./hr.)				
			Per 10 seedlings	Per mg. of embryo	Per 10 seedlings	Per mg. of embryo	Per seedlings	Per embryo	
18	I	0	81.6	21.11	79.7	21.19	1.02	0.99	
		0.25	65.0	16.96	66.0	17.38	0.98	0.98	
		2.5	77.0	17.60	75.3	17.70	1.02	0.99	
	2*	0	76.5	15.51	73.1	14.82	1.05	1.05	
		1	76.4	15.60	70.1	14.30	1.09	1.09	
		5	76.0	15.40	69.3	14.04	1.10	1.10	
		10	75.0	15.20	71.1	14.42	1.05	1.05	
		24	I†	0	144.2	17.36	146.5	17.73	0.98
	0.25			136.5	17.33	133.2	16.61	1.02	1.04
2.5	120.3			15.41	118.3	15.05	1.02	1.02	
5	101.8			12.69	105.8	13.05	0.96	0.97	
30	I	0	243.4	17.46	241.1	17.47	1.01	1.00	
		0.25	197.7	14.03	211.5	15.33	0.93	0.92	
		2.5	179.1	13.09	194.0	14.48	0.92	0.90	
	2	0	173.2	16.42	177.9	16.73	0.97	0.98	
		1	186.3	14.41	194.0	14.74	0.96	0.98	
		5	149.1	10.28	156.4	10.16	0.95	1.01	
	3	0	182.5	15.05	187.8	15.56	0.97	0.97	
		2.5	134.9	12.45	147.8	13.51	0.91	0.92	
	4	0	183.8	16.55	190.3	17.13	0.97	0.97	
		1	200.1	15.36	195.2	15.84	1.03	0.97	
		5	177.8	14.00	180.8	14.46	0.98	0.97	
		10	165.8	10.72	166.4	11.32	1.00	0.95	
	50	I	0	302.2	12.25	323.5	13.33	0.93	0.92
			0.25	269.1	13.11	304.3	14.66	0.88	0.89
			2.5	235.0	11.66	278.1	13.49	0.85	0.86
		2	0	294.2	10.76	307.0	11.41	0.96	0.94
			1	333.4	11.51	336.8	11.70	0.99	0.98
			5	268.8	9.34	294.3	10.05	0.91	0.93
3†		0	399.6	11.71	422.0	12.25	0.95	0.96	
		2.5	379.1	11.22	393.9	11.57	0.96	0.97	
		5	387.5	11.51	392.4	11.50	0.99	1.00	
60	I	0	406.7	12.30	447.0	13.38	0.91	0.92	
		1	389.4	10.67	412.2	11.74	0.94	0.91	
		5	285.9	8.71	379.0	11.24	0.76	0.78	
	2	0	307.9	10.45	344.5	11.33	0.89	0.92	
		0.25	376.1	10.71	402.5	11.14	0.93	0.96	
		2.5	285.0	8.25	288.4	8.33	0.99	0.99	

* Only one determination of O₂ uptake for all treatments in this test.

† Only one determination of CO₂ evolution for all treatments in this test.

lism. This shift, which was less evident for wheat seedlings, has been noted to be typical in previous studies of the metabolism of developing cereal seedlings (4, 10).

On an absolute basis, variation of activities of treated and untreated seedlings of given ages occurred among tests. Some part of this represented experimental error, but part of the variation must have resulted from qualitative and quantitative differences in detail of met-

abolic activity, which were developed in response to environment during culture of seedlings prior to their selection for treatment.

In only four instances—two at 30 hours, one at 50 hours, and one at 60 hours—did the rates (based on ten-seedling samples) of O_2 uptake and CO_2 evolution of wheat treated with 0.25–1 p.p.m. exceed the rate for untreated seedlings. These increases were from 7%

TABLE 6

MEAN RATES OF CO_2 EVOLUTION, O_2 UPTAKE, AND RQ VALUES FOR DUPLICATE SAMPLES OF MUSTARD SEEDLINGS TREATED FOR 3 HOURS WITH 0.25–10 P.P.M. OF 2,4-D. VOLUME RATE FOR UNTREATED SAMPLE VALUED AS 100 RELATIVE TO RATE FOR TREATED SAMPLES IN EACH TEST

TEST NO.	SEEDLING AGE (HR.)	PRETREATMENT TIME (HR.)	2,4-D TREATMENT (P.P.M.)	ACTIVITY OF UNTREATED SEEDLINGS (CU. MM./HR./10 SEEDLINGS) AND RELATIVE ACTIVITY FOR TREATED SEEDLINGS		RQ
				CO_2	O_2	
33	30	3	0	40.2	42.6	0.94
			0.25	91	91	.94
			5	82	84	.92
35	30	3	0	33.3	39.2	.85
			1	97	91	.90
			10	86	89	.88
13*	50	3	0	93.5	136.8	.67
			2.5	89	94	.66
			10	87	90	.67
37	50	3	0	107.3	128.0	.84
			1	86	89	.80
			5	88	91	.81
34†	50	3	0	92.3	95.0	.97
			0.25	89	91	.94
			2.5	86	90	.93
15†	75	3	0	81	81	.96
			2.5	145.7	199.1	.73
			10	79	94	.62
14*	70	24	0	77	88	.64
			2.5	140.4	188.5	.75
			10	99	96	.78
				97	95	0.76

* Tests 13 and 14 were conducted as a series in which length of pretreatment time varied as indicated. CO_2 evolution rate was measured only on one sample in these tests.

† Three determinations of rate of O_2 uptake and one of CO_2 evolution made for each treatment in this test.

to 22% and were greater in the older than in the younger seedlings. The variation among rates for replicates in a test of older seedlings (8% or less when seedlings were 50 hours old or more) was usually greater than that (less than 5%) which occurred in tests of younger seedlings. When the data were calculated on the basis of unit weight of embryonic tissue, five of the apparent increases proved to be decreases, and the other increases were of lesser magnitude.

By far the main effect of 2,4-D was to reduce all rates of activity in proportion to the concentration applied. Some unexplainable variations from the smoothness of this general trend appeared for both wheat and mustard seedlings. The main difference between the responses of the two kinds of treated seedlings concerned the degree of changes in rates of O_2 uptake or of CO_2 evolution. The O_2 uptake of wheat was generally decreased by 2,4-D relatively more than that of mustard. This difference was irregular and not great.

The rate of CO_2 evolution of mustard was decreased in degree approximately equal to, or greater than, the reduction in the rate of O_2 uptake (table 6). Decreases in the rates of CO_2 evolution of wheat which occurred when low concentrations (0.25–2.5 p.p.m.) were applied to seedlings of different ages in several of the tests were relatively slightly less than the decreases in rates of O_2 uptake (table 5). In general, as a result of these responses the RQ values for treated mustard usually tended to remain similar to those of controls, or to decrease, while the RQ values for treated wheat increased slightly in several instances, or were similar to those of untreated seedlings.

For both kinds of seedlings of all ages, treatment with 5–10 p.p.m. of 2,4-D

caused decreases in the rate of CO_2 evolution approximately equal to, or greater than, the marked decreases in the rate of O_2 uptake. The RQ values often were lower than controls. Evidence has been presented (12, 18) that anaerobic metabolism is normally stronger during the period of early germination than during the later development of some species. The results of all the tests on wheat and mustard suggest stronger anaerobic metabolism in wheat than in mustard. Though responses were sometimes irregular, a comparison of the complexes of responses in younger and older seedlings, especially wheat, seems to indicate somewhat stronger anaerobic metabolism during the earlier stages of development.

Discussion

Soon after several different chemical growth-regulators, in particular 2,4-dichlorophenoxyacetic acid (7, 13, 21, 26), are sprayed on vegetative tops, responses in parts of several species of dicotyledonous plants appear to involve mobilization of reserve carbohydrate, increase in content of soluble sugars, and increase in metabolic activity, often followed by decrease in soluble sugars and by the development of malconditions or by the death of the plants (2, 13, 15, 22). Hydrolyses of reserves and their mobilization over relatively short distances from seed-storage organs to the rapidly growing and metabolizing embryonic parts normally occur at high rates in seedlings (1, 4, 10). Germinating seedlings contain appreciable amounts of soluble sugars (1).

Many kinds of seedlings have been shown to be relatively highly sensitive to treatment with 2,4-D (3, 8, 16, 25). Development of malconditions, often death, frequently has been shown to follow soon after treatment of seedlings with relatively low concentrations of the acid. Al-

though growth was somewhat inhibited, O_2 uptake by wheat seedlings was stimulated by low concentrations of indoleacetic acid (19). This acid stimulated O_2 uptake and protoplasmic streaming in oat coleoptiles when applied in concentrations of 10 p.p.m. or less (5, 23).

There was very little, if any, distinct indication of any increase in metabolism of energy liberation in seedlings after treatment with 0.25–10 p.p.m. of 2,4-D. Usually such activity decreased soon after treatment. Results of tests of oxidative processes in seedlings and of tests on established plants (7, 21) would seem to indicate another instance of marked difference in response of tissues to treatment with 2,4-D.

In so far as judgment can be based on evidence from gas-exchange studies, the results of tests on wheat and mustard seedlings indicated that metabolism was decreased by treatment with 0.25–10 p.p.m. of 2,4-D. In general, the apparent responses of the monocotyledonous and dicotyledonous species appeared similar, even though reports of investigations on seedlings have clearly shown a differential herbicidal effect of 2,4-D on various plant groups and varieties (3, 8, 16, 25).

At least in the case of wheat and mustard, the differential herbicidal effect does not seem to be primarily concerned with a distinct difference in response of the energy metabolism of the seedlings. Nor does it appear likely, on the basis of gas-exchange responses, that abnormal, rapid depletion of metabolic and growth substrate, induced by treatment with 2,4-D, would be a major factor possibly contributing toward development of starvation as a part of the complex of malconditions reported to occur in seedlings after treatment.

HSUEH and LOU (9) recently have reported that metabolic activity and germination of seedlings were distinctly decreased and delayed, or completely inhibited, during tests lasting 6 days when seeds of a number of species were treated with relatively high concentrations (up to 1000 p.p.m.) of 2,4-D. In general, the gas-exchange responses which they observed for treated seed, especially barley, seemed qualitatively similar to those which are here described for wheat and mustard seedlings treated with 10 p.p.m., or less, of the acid. They found that barley, wheat, and seed of a number of dicotyledonous species did not germinate when treated. Rice seed showed germination activity of some nature which was somewhat delayed compared with that of untreated seeds. Mainly by means of manometric tests they found that apparent anaerobic oxidation in rice was engendered by 2,4-D. This response seemed related to germination activity in rice. The tenable, but not entirely proved, suggestion was made that the anaerobic oxidation processes in rice treated with 2,4-D were able to supply energy required for endothermic processes of germination.

In considering the differences observed in the relative amounts of germination of rice compared with other seeds tested by HSUEH and LOU, it is interesting to note the relative rates of respiration. The apparent aerobic activity of rice after treatment was greater than that of other similarly treated seedlings. It seems possible that the greater development by treated rice seedlings could as well be correlated with their higher level of aerobic respiration.

The difficulty one experiences in following treatment by HSUEH and LOU of their data precludes the possibility of any

direct comparison between results of their manometric tests and those of others. Moreover, their manometric data are difficult to evaluate critically because quantitative expression of volume rates of gas exchange or the constants to allow calculation of their data in terms of volume rates were not given. It did not appear that the progressive development of the effect of treatment on growth (volume and uniformity of samples) was given detailed consideration in the evaluation of the results of their manometric tests.

In some tests conducted by the writer in an atmosphere of 100% nitrogen, the rate of CO₂ evolution by rice seedlings was slightly greater in 2.5 p.p.m. of 2,4-D than for controls during a period of approximately 6 hours after initiation of treatment of seedlings 30 hours old. The rate for seedlings treated with 2,4-D then decreased and was distinctly less than that of anaerobic controls before the twenty-fourth hour of treatment.

In somewhat the same manner as do anaerobic environments or treatments with oxidase inhibitors (1, 12, 24), treatment with 2,4-D does appear to elicit the expression of anaerobic activity in treated tissues in which potentials for such activity exist. Part of the results of reported studies on the influence of 2,4-D on the metabolism of energy release can be interpreted to indicate this effect in some degree.

Summary

1. Seedlings of wheat and mustard were selected when 18–24, or more, hours old and were treated with 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations up to 10 p.p.m. Manometric measurements of the rates of CO₂ evolution and of O₂ uptake were made at various intervals from the first through the twenty-fourth hour after treatments were initiated. A number of tests were conducted in which the activities of seedlings 18–70 hours old were measured after these had been exposed for 3 hours to 2,4-D treatments.

2. The main responses were reduction in rates of CO₂ evolution and of O₂ uptake by both species at all ages. Apparent reduction of activity, generally in proportion to concentration of 2,4-D, was measured during the first hour after initiation of treatment, and such reduction increased with duration of treatment.

3. In a number of tests, especially on wheat the rate of CO₂ evolution was decreased less than the rate of O₂ uptake, so that *RQ* values of treated seedlings were slightly higher than those of controls. These responses, which appeared more irregularly and less distinctly for mustard than for wheat and which appeared somewhat more frequently in earlier stages of development of seedlings, are discussed in relation to the herbicidal effect of treatment with 2,4-D.

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EFFECTS OF SEVERAL SULFA-COMPOUNDS ON NUCLEAR AND CELL DIVISION¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 593

THOMAS C. FULLER

Introduction

Since the discovery that polyploidy can be produced in plants almost at the will of the investigator through the use of colchicine (2, 3, 4), considerable interest has developed in the possibilities thus opened up, and experiments with other chemicals have been going on. More recently EIGSTI (5) reported that "mitotic irregularities" were induced in the pollen tubes of *Tradescantia occidentalis* when they were treated with dilute concentrations of sulfanilamide (1:10,000). Greater concentrations inhibited the formation of the tube. TRAUB (9) found polyploid (4n) and numerous binucleate cells in *Allium* roots after 48 hours of treatment with sulfanilamide (0.5%). PETERS (8) reported that it produced an extreme contraction of the chromosomes of *Allium* at metaphase and inhibited the spindle mechanism. He noted that after reaching the metaphase stage, the chromosomes reverted to an active metabolic condition without completing the mitotic cycle. The reversions occurred both before and after the division of the centromeres, resulting in the production of tetraploid nuclei which were observed in blocked metaphases as early as 8 hours after treatment. He also found that sulfanilamide inhibited the entrance of cells into mitosis and that divisions rarely occurred after 48 hours of treatment. Although less effective than colchicine in

inducing polyploidy in *Allium* (8), sulfanilamide has been more effective in some species of *Vaccinium* (6).

These reports led to a reinvestigation of the effects of sulfanilamide on nuclear and cell divisions, together with the effects of several other sulfa-compounds: viz., sulfadiazine, sulfaguanidine, sulfamerazine, sulfapyridine, and sulfathiazole.

Material and methods

The bases of bulbs of *Allium cepa* L. were placed in tap water in Stender jars in the laboratory for a period of 24-48 hours until roots averaging approximately an inch in length were developed. These roots, while still attached to the bulbs, were treated by immersing them in aqueous solutions of the sulfa-compounds.

Preliminary tests of the effects of sulfanilamide were made in solutions of 0.125, 0.25, and 0.5% concentration by weight in tap water at 20° C. Because the greatest effects occurred in the 0.5% solution, the weaker concentrations were not used in the later experiments. All the other sulfa-compounds were used at 0.1% concentration by weight in tap water because of their low solubility. Even this quantity was slightly above the saturation point of these compounds in water, and a slight excess of solid material was present in each solution.

Treatments were started at 12:30 P.M., and the solutions were changed every 24 hours. Root tips were collected

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at intervals up to 96 hours following the start of the treatments. After 48 hours some treated roots were returned to tap water, and collections were made from them 24 and 48 hours later. A few treated roots were also returned to tap water at the end of 96 hours, but, since they showed essentially the same responses as those returned to tap water after 48 hours of treatment, this procedure was not repeated in later experiments. Control bulbs were continued in tap water, which was also changed at 24-hour intervals. Both treated and control root tips were fixed in Navashin's solution, handled according to the tertiary-butyl-alcohol method, and imbedded in paraffin. Sections cut transversely at $20\ \mu$ were stained with Heidenhain's iron-alum hematoxylin with a counterstain of Orange G in clove oil.

Results

The first observable response following the immersion of the roots in the solutions of the sulfa-compounds was the cessation of visible elongation, and it was not renewed during the 96-hour period of treatment. The roots also became progressively discolored with a brownish tinge during treatment.

Approximately half the roots immersed in 0.5% sulfanilamide showed an increase in diameter in the morphological region of elongation within 24 hours. The enlargements reached a maximum size within 48 hours after treatment as a result primarily of an increase in size of the cortical cells. Similar tumors were not formed following treatment with any of the other sulfa-compounds except for one bulb in which an initial swelling was noticed in approximately half the roots after 72 hours of immersion in the sulapyridine solution. These tumors, comparable to those from the sulfanilamide

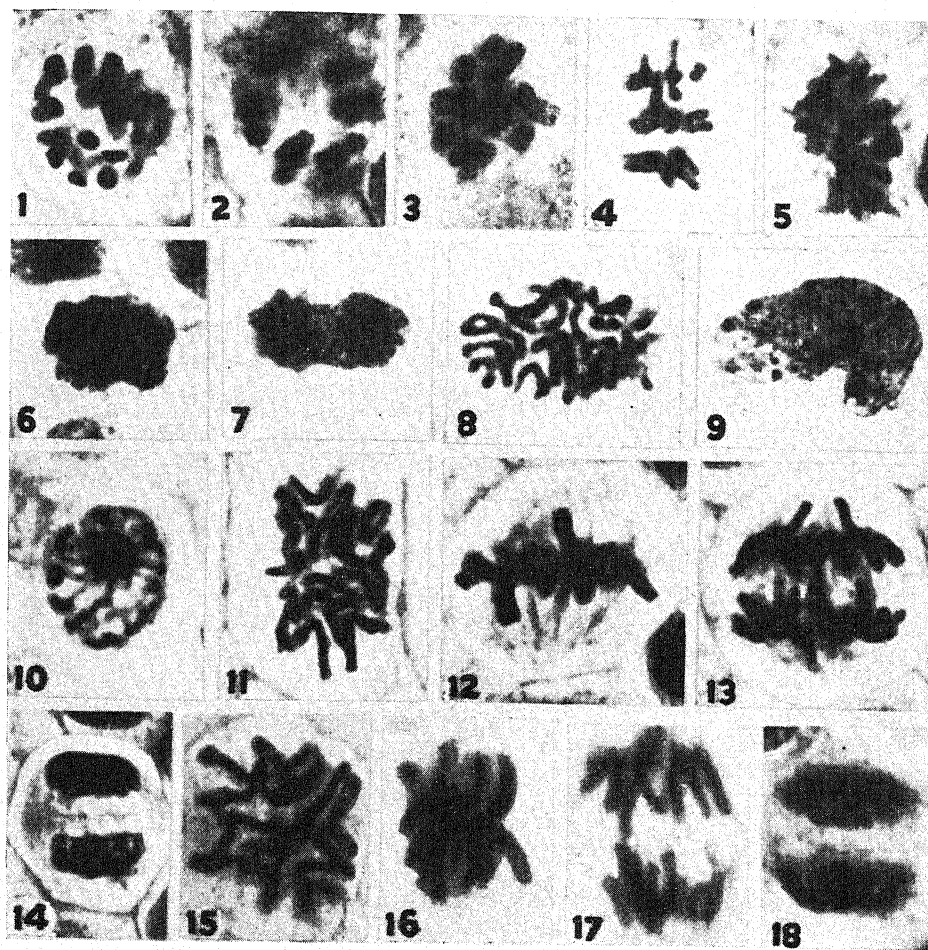
treatments, reached a maximum size at the end of 96 hours. On being returned to tap water, all the treated roots, including those showing tumors, resumed growth. The new growth was noticeably whiter than the darkened older portion.

In a series of preliminary experiments with sulfanilamide at 0.125, 0.25, and 0.5% concentration in tap water at 20°C ., the elongation of roots in the two lower concentrations was inhibited, but tumor formation did not occur. Cells from these roots showed late prophase and metaphase stages with slightly shortened chromosomes and a much slower rate of division than in control roots. At 0.5% concentration sulfanilamide had a more pronounced effect on mitosis. Preliminary examinations of roots at hourly intervals up to 10 hours following the start of treatment showed a progressive shortening of the chromosomes at the late prophase and metaphase stages up to 3 hours, when the maximum contraction appeared to be reached. Because the changes occurred very slowly, later examinations were made at 6, 12, 24, 48, 72, and 96 hours after immersion in the solution.

Mitosis was found to occur in a few cells throughout the 96-hour period of treatment but at such a slow rate and in so few cells that no visible elongation of the treated roots took place during this period. The prophase nuclei responded to sulfanilamide by developing unusually contracted and condensed chromatids. In early prophases the chromosomes were scattered throughout the nucleus. Spiralization of the chromatids could rarely be observed, since it was somewhat obscured by condensed, densely stained material. While still in the mid-prophase stage, the chromosomes were markedly contracted (fig. 1). In later prophases they were scattered around

the inner periphery of the nuclear membrane, the center of the nucleus being clear (fig. 2). In one series of treatments no prophase stages were observable at 48 hours. This occurred under the same experimental conditions as in the other treatments but was not observed again.

The metaphase figures containing the much shortened chromosomes appeared flat when compared with the same stages in the controls (figs. 3, 15). The chromatids were characteristically parallel to each other throughout the metaphase and remained thus until disruption of the



FIGS. 1-18.—Figs. 1-9, cells after treatment with 0.5% sulfanilamide: fig. 1, prophase, 12 hours; fig. 2, late prophase with shortened chromosomes, 12 hours; fig. 3, metaphase with shortened chromosomes, 12 hours; fig. 4, metaphase with disrupted spindle, 48 hours; fig. 5, early reversion stage, metaphase, 96 hours; fig. 6, reversion stage, metaphase, 48 hours; fig. 7, reversion stage, anaphase, 48 hours; fig. 8, metaphase, tetraploid, 96 hours; fig. 9, lobed nucleus from cortical cell of tumor, 96 hours. Figs. 10-14, cells treated with other sulfa-compounds: fig. 10, prophase, sulfathiazole, 12 hours; fig. 11, metaphase plate, sulfadiazine, 6 hours; fig. 12, metaphase, side view, sulfapyridine, 96 hours; fig. 13, anaphase, sulfapyridine, 96 hours; fig. 14, telophase, sulfamerazine, 48 hours. Figs. 15-18, controls, cells untreated: fig. 15, metaphase plate; fig. 16, metaphase, side view; fig. 17, anaphase; fig. 18, telophase.

spindle mechanism occurred. This breakdown first became evident when the metaphase figure was no longer flattened. Concurrently, the chromatids were repelling each other strongly even before the division of the centromeres (fig. 4). The centromeres appeared to divide, however, before the chromosomes reverted to the metabolic condition. Reversion stages were found in material at 48–96 hours (figs. 5, 6). A number of anaphases in which the spindle mechanism had disrupted and in which the chromosomes were reverting to the metabolic state without completion of the mitotic cycle were found at 48 hours (fig. 7). These cells may possibly have been in a late metaphase or anaphase condition at the beginning of treatment. Binucleate cells resulting from the failure of cell-plate formation were also present; probably these cells had been in a late anaphase or telophase stage when treatment was started, and the consequent breakdown of the spindle mechanism resulted in their development. Metaphase and anaphase figures with the tetraploid number of chromosomes were found 96 hours following the application of sulfanilamide (fig. 8), but no other polyploidy was found.

The greatly enlarged cortical cells of the tumors had a large central vacuole, but their nuclei appeared similar to those of the controls. A few nuclei, in the metabolic condition, were much larger and were possibly tetraploid. A few of the greatly enlarged cells showed lobed nuclei resembling those of certain secretory cells or cells of maturing xylem vessels (fig. 9).

After immersion in 0.5% concentration of sulfanilamide for 48 hours, some bulbs of each series were transferred to tap water. No tetraploidy was found in cells from these roots at 24 and 48 hours after being returned to tap water.

Roots treated with the other sulfam compounds—sulfadiazine, sulfaguandine, sulfathiazole, sulfamerazine, and sulfapyridine—all showed the same general responses. Although there was no visible elongation following the application of the compounds, all stages in the mitotic cycle were found at all intervals during treatments, but with the division rate markedly retarded. The prophase and telophase stages appeared similar to those in controls. The metaphase and anaphase figures showed shortened chromosomes, but the degree of contraction was less than that resulting from treatment with 0.5% sulfanilamide, although similar to that of chromosomes in cells treated with the weaker concentrations of sulfanilamide. The principal effect was the marked slowing of the rate of divisions.

Roots of the one bulb that produced tumors in the sulfapyridine solution failed to show the cytological effects resulting from 0.5% sulfanilamide, except for the enlargement of the cortical cells. The mitotic figures were similar to those in roots from the other bulbs treated with sulfapyridine which developed no tumors.

Discussion

The results of this investigation confirmed the previous reports (8, 9) of the gross effects of sulfanilamide in that elongation of the roots ceased and tumors were developed in the morphological region of elongation. In the present experiments tumors were never formed on more than half the roots of any bulb. No visible differences could be detected between the nuclei of roots that formed tumors and those that did not, whether treated or untreated, except that an occasional nucleus in the tumor showed a lobed outline. Tumor formation did not occur following treatment with the other

sulfa- compounds, except in one bulb treated with sulfapyridine. Enlargement of the cortical cells to form tumors was therefore probably a result of a physiological disturbance in these cells.

All the sulfa- compounds prevented visible elongation of roots, while those of the controls grew to the bottom of the containers during the same time interval. Mitosis occurred in some cells of all the treated roots, except for one series of treatments with sulfanilamide in which mitosis was completely blocked at 48 hours. This agrees with the results of PETERS (8), although complete blocking of mitosis was, however, not again observed in any of the four repetitions with sulfanilamide.

The results with 0.5% concentrations of sulfanilamide were generally the same as those found by PETERS except that the responses were slower than he reported. Reversion stages were found at 48-96 hours after treatment began. PETERS reported them as early as 3 hours after treatment, with blocked metaphases in tetraploid nuclei as early as 8 hours. Only after 96 hours of treatment were tetraploid nuclei observed in the present experiments. In some of these the chromosomes appeared in flattened metaphase plates, although others showed apparently normal anaphase configurations during the time in which the roots were still immersed in the sulfanilamide solution.

According to PETERS, the prophase stages in treated roots were similar to those of the controls, with the chromatids a little more distinct. In the writer's material the chromatids appeared somewhat obscured by a slight cloudiness around them during the early prophase stages and were therefore less distinct than in the controls. In later prophase stages, however, the chromatids were more distinct than in the controls. Dur-

ing the late prophase stages the chromosomes may be as much shortened as they become in any of the metaphase stages. These latter stages were similar to those resulting from colchicine treatment, according to figure 4 of BERGER and WITKUS (1) except that this figure showed no nuclear membrane. They interpreted this as representing a disruption of the spindle mechanism at metaphase, with the chromosomes grouped around an achromatic sphere. PETERS reported that, following such a grouping, reversions to an active metabolic condition occasionally occurred. In the present experiments all the chromosomes appeared to reach the metaphase-plate stage before there was any indication of the disruption of the spindle. It seems probable that a breakdown of the nuclear membrane in a late prophase, when the short highly condensed chromosomes are arranged peripherally around the clear center of the nucleus, would result in the appearance described. Thus, what PETERS and BERGER and WITKUS interpreted as a type of disruption of the metaphase may instead be a late prophase in which the nuclear membrane had been dissolved and in which the chromosomes were reverting to an active metabolic condition without first being arranged in a metaphase plate.

The marked differences in results of treatment with sulfanilamide (0.5% concentration) and of those with the five other sulfa- compounds (0.1% concentrations) were possibly correlated with the lower solubility in water of the latter. This conclusion would be in accord with the findings of ÖSTERGREN and LEVAN (7), who used benzene, cyclohexane, and thiophene and various halogen derivatives of these compounds—monobromobenzene, monochlorobenzene, bromocyclohexane, dibromothiophene, etc.

No explanation can at present be offered for the more rapid results obtained by PETERS with 0.5% solutions of sulfanilamide than the consistently slower effects observed during the course of this investigation.

For demonstrating mitosis to the beginning student it is suggested that onion roots be immersed for 6 hours, before being fixed, in a 0.1% concentration of one of the sulfa- compounds. Under these conditions all stages of the mitotic cycle are essentially normal except that the chromosomes are thicker and shorter and therefore less entangled. Flat metaphase figures are obtained in which the sixteen chromosomes are clearly visible and in which the chromatids also show more clearly in the late prophase than in untreated material.

Summary

1. Onion roots approximately 1 inch long were immersed in solutions of 0.5% sulfanilamide and of 0.1% in tap water of the following sulfa- compounds: sulfadiazine, sulfaguanidine, sulfamerazine, sulfapyridine, and sulfathiazole. These solutions were at or slightly above the maximum solubility of these compounds in water at 20° C. The solutions were changed every 24 hours, as were the controls in tap water. Fixations of the roots were made after 6, 12, 24, 48, 72, and 96 hours of treatment. Some bulbs from each series were returned to tap water after 48 hours and others after 96 hours.

2. There was immediate cessation of visible root elongation in all the treatments, but approximately half the roots of the bulbs treated with sulfanilamide developed tumors within 48 hours, owing to enlargement of the cortical cells in the region of elongation. In only one other treatment were such tumors formed—on about half the roots of one bulb immersed in sulfapyridine.

3. In roots treated with sulfanilamide normal-appearing mitosis continued in a small number of the cells of the cortex and apical meristem throughout the 96-hours period of treatment. The duration of the mitotic process seemed to be materially lengthened. Disruption of the spindle mechanism and delay in the separation of the centromeres of the chromosomes were observed. As a result of the inhibition of the spindle mechanism, reversion stages occurred which resulted in the return of the anaphase chromosomes to the metaphase condition without completion of the mitotic cycle and in the production of tetraploid metaphase and anaphase figures within 96 hours after application of sulfanilamide. In no case, however, was a tetraploid cell found in division after normal growth had been resumed in tap water. Binucleate cells, resulting from the failure of the formation of the cell plate, were observed after 48 hours of treatment. The nuclei of the cortical cells in the enlarged regions appeared normal in most cells, although some nuclei were much larger than others and than those of the controls. These large nuclei were probably tetraploid. A few other nuclei showed irregular, lobed outlines. No micronuclei were found, in contrast to the report of PETERS. The only polyploidy observed was tetraploidy.

4. Mitosis was completely blocked at 48 hours in one series of treatments with sulfanilamide. In these roots no mitotic activity and no characteristic tumor formation occurred.

5. The other five sulfa- compounds induced essentially the same responses in all the roots. Although there was no visible elongation, mitosis nevertheless continued at a slow rate throughout the 96 hours of treatment. Even though the chromosomes were shorter than in the controls, the degree of contraction was

not so great as had occurred in cells treated with sulfanilamide. On return to tap water all treated roots resumed normal growth.

6. The markedly different responses to sulfanilamide and to the other sulfa-compounds occur possibly because of the greater solubility in water of sulfanilamide than of the others.

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FORMATIVE EFFECTS OF CERTAIN SUBSTITUTED CHLOROPHENOXY COMPOUNDS ON BEAN LEAVES¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 594

DANIEL F. BURTON

Introduction

One of the most striking effects of the application of substituted phenoxy compounds to plants is the induction of leaf modifications. ZIMMERMAN (10, 11) has stressed the importance of the study of modifications in form induced by the hormone-like substituted phenoxy compounds by pointing out that protoplasm is capable of a variety of reactions and that it is desirable to investigate any

mechanism that determines which of the potential reactions actually shall occur. He also suggested that, since form is determined by cell behavior, the substituted phenoxy compounds and the substances secreted by the nucleus may control cytoplasmic activity in a similar fashion and hence control form.

FOSTER (5) has indicated the paucity of data on leaf histogenesis compared with analogous data on stems and roots. Therefore, in the present experiments the normal growth pattern in leaves of the Red Kidney bean was examined as well

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as the growth patterns induced by the application of growth-regulating substances.

Material and methods

Red Kidney beans were planted in flats containing fertile loam soil and placed on greenhouse benches for germination. When the primary heart-shaped leaves were approaching their mature size and the first trifoliate leaves were starting to emerge from the bud, the flats were divided into four groups. The plants in the first group were treated with 0.5% 2-chlorophenoxyacetic acid in Carbowax 1500 applied to the upper surface of the blade near the bases of the primary leaves, in amounts estimated to contain 40 mg. of the mixture per plant, or 200 γ of the acid (3). The second, third, and fourth groups were treated with 4-chlorophenoxyacetic acid in lanolin, 2,4-dichlorophenoxyacetic acid (2,4-D) in lanolin, and 2,4-dichlorophenoxyacetic acid in Carbowax, respectively, in the same concentrations and in amounts approximating those used in the first group. Some plants in each flat were left untreated as controls.

Collections of buds, young leaves, and fully developed leaves were made from treated plants and controls. The material was fixed in Navashin's solution, handled according to the tertiary butyl-alcohol method, sectioned at 10-15 μ , and stained with KRAUS's modification of Fleming's triple stain.

Three sets of beans were planted at Chicago. The first set was planted on March 27, 1946, treated on April 6, and leaves and buds were collected on May 9. The second set was planted on April 11 and treated on April 22. At the time of treatment of this set, temperatures in the greenhouse were unseasonably high, and so many of the plants died that no

collections were made. A third set was planted on May 21, treated on June 1, and collections were made on June 22. A fourth set, planted at State College, Mississippi, on March 26, 1947, was treated only with 2,4-D in lanolin and in Carbowax, on April 8; collections were made on April 29.

Observations

DEVELOPMENT AND APPEARANCE OF NORMAL TRIFOLIATE LEAVES.—The internal tissues of the lamina of a normal leaflet originate by the activity of sub-epidermal marginal meristems which are found on the lateral edges of the embryonic midrib nearer the adaxial surface (fig. 2A). Divisions of cells of these meristems give rise to four layers of cells between the upper and lower epidermis (fig. 2B). These layers plus the upper and lower epidermis constitute the plate meristem. The cells of the adaxial layer of internal tissue are larger than those of the other internal layers. Since the six cell layers in the lamina of the embryonic leaflet are still present in the lamina of a leaflet just prior to the development of intercellular spaces, all division of cells in the plate meristem, except divisions involving veins, must produce daughter cells in the plane of the plate meristem.

Initial cell divisions in the development of veins of the minor vascular network produce daughter cells vertical to the plane of the plate meristem. Such divisions occur in a single row of cells in the layer immediately beneath the layer of enlarged cells. In cross section, this row appears as a single cell. Figure 2C, illustrating a section cut obliquely to the row, shows a cell in which one such division has occurred, giving rise to the first two cells of the veinlet. To the right of these cells there were two divisions, producing three cells. Figure 2D shows a later stage

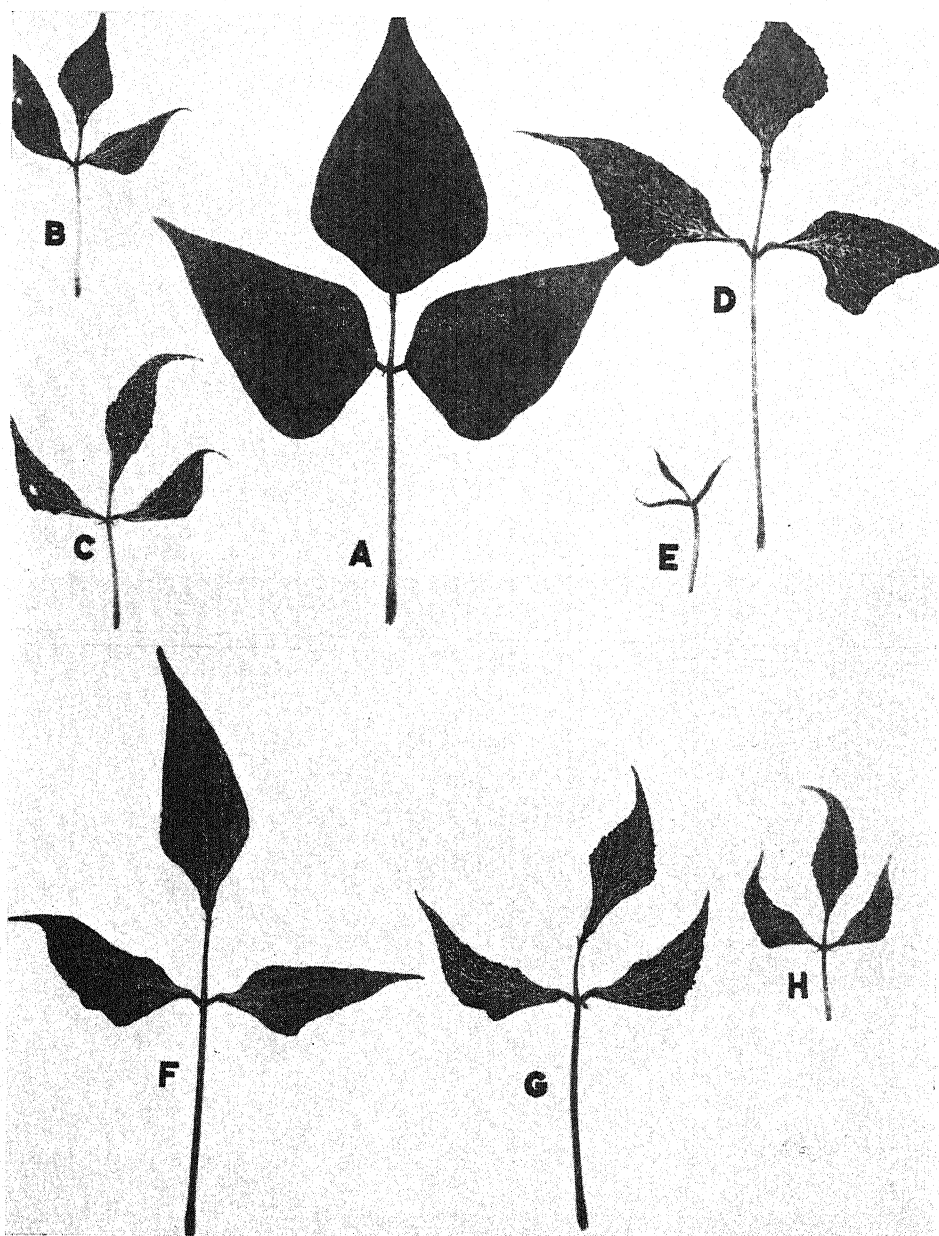


FIG. 1.—Trifoliate leaves from control (A) and treated plants (B–H). Plants treated with (B) 2,4-D in Carbowax 1500; reduced size and increased prominence of veins compared with control; (C) 2,4-D in lanolin; effects similar to B; (D) 2-chlorophenoxyacetic acid; leaflets reduced and prominent veins similar to C; (E) 4-chlorophenoxyacetic acid; narrow straplike leaflets ultimately produced; (F, G, H), 2,4-D in lanolin; leaves taken in succession to illustrate limitation in size of later leaves.

in the formation of smaller veins. An adjacent cell of the same layer has divided, as have several cells in the next lower layer.

A section through the same leaflet as that shown in figures 2*A* and 2*B* is illustrated in figure 2*E*. Although the tissues at the level of figures 2*A* and 2*B* were embryonic, those at the tip of the leaflet were mature, thus demonstrating that

maturation begins at the tip. When fully expanded, the leaflet has a single layer of palisade parenchyma beneath the upper epidermis (fig. 3*A*), derived from the upper internal layer of enlarged cells illustrated in figures 2*A*, *B*, *C*, and *D*. The spongy mesophyll is derived from the other three internal layers (fig. 3*A*).

The vascular elements of the midrib (fig. 3*A*) and the largest veins are sur-

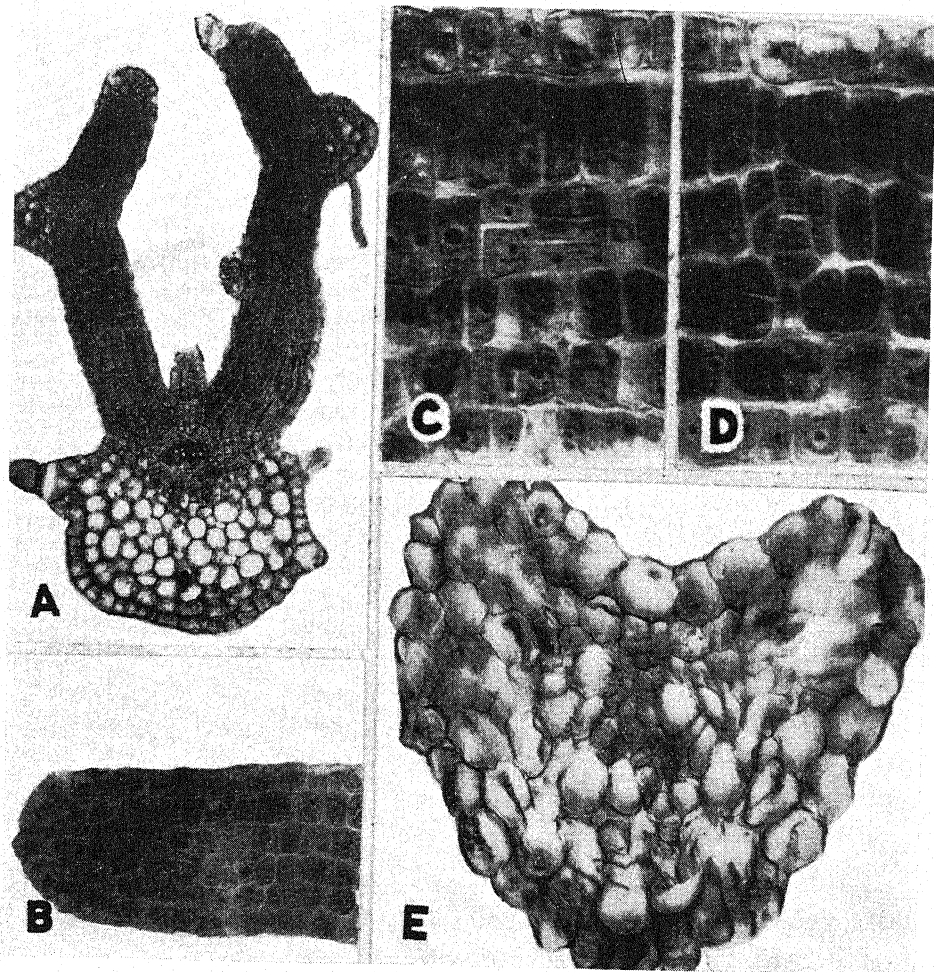


FIG. 2.—Embryonic leaflet of control. *A*, cross section through leaflet; lamina arising from embryonic midrib. *B*, detail of marginal and plate meristems shown in *A*. *C*, oblique section through row of cells initiating a vein in upper layer of spongy-mesophyll plate-meristem. *D*, cross section through similar row of cells, illustrating more advanced stage of vein formation. *E*, tip of leaflet, showing maturity of tissues at this region of same leaflet shown in *A* and *B*.

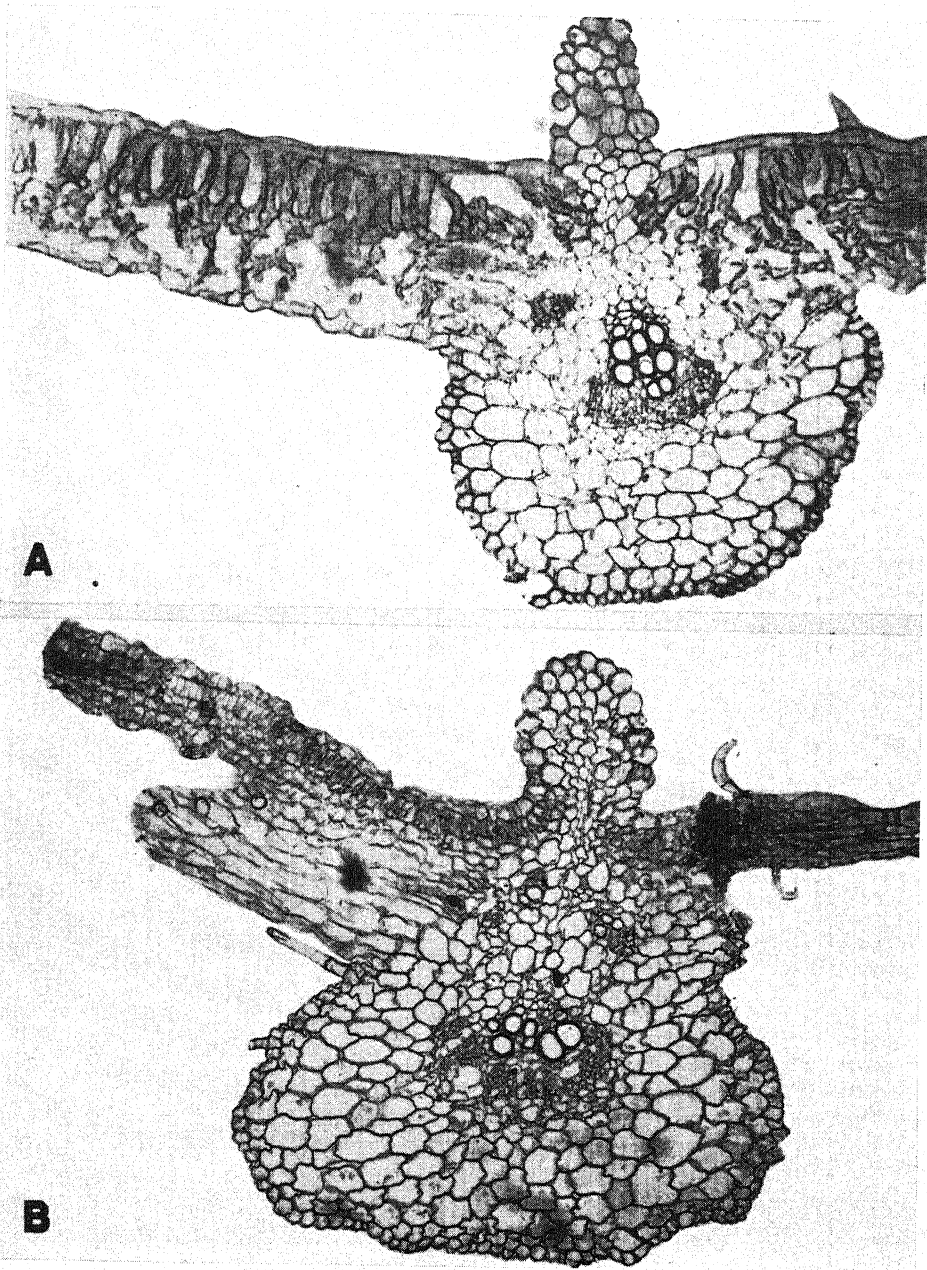


FIG. 3.—Cross section through mature leaflet in region of midrib of: (A) control with well-developed intercellular spaces in mesophyll and extensive region of parenchyma around vascular elements; (B) plant treated with 2-chlorophenoxyacetic acid; normal vascular tissue but no intercellular spaces in mesophyll.

rounded by an extensive parenchymatous bundle sheath which contains no chloroplasts. Smaller veins have a less extensive sheath, and in the smallest veins the sheath is only a single layer of parenchymatous cells often containing chloroplasts.

The mature leaf is trifoliate. The lamina of a leaflet is marked by a network of fine anastomosing veins (fig. 1A). A stipule subtends each of the lateral leaflets, with an additional pair beneath the terminal leaflet. Stipules persisted in the normal position in treated plants.

GROSS EFFECTS OF TREATMENTS.—Little or no epinasty or stem curvature occurred in plants treated with 2-chlorophenoxyacetic acid. At first the growth of these plants was comparable with that of controls, and the first few trifoliate leaves were essentially normal. Leaves produced later were smaller and had darker green leaflets with light-colored veins which, though less numerous, were more prominent than the veins of controls (fig. 1D).

The plants to which 4-chlorophenoxyacetic acid was applied showed pronounced epinasty and stem curvature in a few hours. Curvature frequently turned the stem through 180° , so that it appeared to have reversed from negative to positive geotropism. In the second lot, treated during a period of high temperatures, 80% of the plants were dead in 17 days. If a plant was not killed, its tip eventually resumed an upright growth pattern; by this return to the normal orientation an S-curve was produced in the hypocotyl and first internode. The first trifoliate leaf produced on a partly recovered stem was similar in appearance to the modified leaves produced on plants treated with 2-chlorophenoxyacetic acid. This leaf was just starting to emerge

from the bud at the time of treatment. Similar but smaller leaves were sometimes developed subsequently, but most leaves formed later were narrow and straplike (fig. 1E). In such leaves the unpigmented midrib was wider than that of normal leaflets and often composed four-fifths of the total width of such anomalous structures. No other venation was apparent. The margins of the straplike leaflets were rolled and green.

Until leaves began to develop, the behavior of plants treated with 2,4-D, in either carrier, was similar to that of plants treated with 4-chlorophenoxyacetic acid. Epinasty and stem curvature occurred more rapidly and sometimes more severely in plants treated with 2,4-D than with either of the other two acids. All plants in the second lot to which 2,4-D had been applied in lanolin died within 17 days. When Carbowax was the carrier, most of the plants were alive after 17 days, but there were so few signs of recovery that all were discarded.

In those lots in which plants treated with 2,4-D showed partial recovery, the first few leaves produced resembled the modified leaves on plants treated with 2-chlorophenoxyacetic acid (figs. 1B, C). Leaves produced later were even smaller than earlier leaves on the same plant (figs. 1F, G, H). The most extremely modified leaves had midribs and veins fasciated into a wide band of unpigmented tissue, although the margins and occasional islets in the fasciated bands were green.

HISTOLOGICAL RESPONSES.—The modified leaves of plants treated with 2-chlorophenoxyacetic acid developed much like those of controls until the stage at which intercellular spaces normally would have appeared. Expansion of chlorenchymatous layers then ceased, and no intercellular spaces were formed

in either the palisade or spongy mesophyll (fig. 4A). Since the cells of these tissues contained chloroplasts, there was a greater amount of green pigment in a given volume of the leaflet than in a like volume of a normal leaflet containing intercellular spaces. There was no histological evidence of changes in the midrib or major veins (fig. 3B), so that the increased prominence of these veins in gross aspect was probably a result of contrast with the darker green mesophyll. The darker color of the latter probably helped to obscure the minor veins, since they were not surrounded by an unpigmented parenchyma extending from the lower to the upper epidermis.

Laminae of leaflets from plants treated with 2,4-D developed internal structures by the activity of marginal meristems similar to the marginal meristems of controls (fig. 5A). These meristems laid down four internal layers of plate meristem (fig. 5B) similar to the corresponding layers in the controls. Thus, the six layers of plate meristem described for the embryonic leaves of controls were present in the embryonic leaves of treated plants.

As in the controls, maturation of tissues began at the tip of a leaflet. Figure 4B shows a cross section through the mature tissues at the tip of the same leaflet which had embryonic tissue at the level shown in figures 5A and 5B. The tissues of mature laminae of the earliest-formed leaflets of plants treated with 2,4-D resembled closely the comparable tissues from leaflets of plants treated with 2-chlorophenoxyacetic acid. In the later-formed leaves of plants treated with 2,4-D, fasciation of the veins was extensive. The vascular elements were discrete, but the unpigmented parenchyma surrounding the vascular elements of the midrib and major veins was continuous

through much of the width of the leaflet. Chlorenchymatous tissue was confined to the margins and occasional islets surrounded by fasciated veins. There were no intercellular spaces in the chlorenchyma. Figure 5C shows a cross section from an extreme example of the smaller leaflets.

In the narrow, straplike leaflets of plants treated with 4-chlorophenoxyacetic acid, fasciation of the veins was

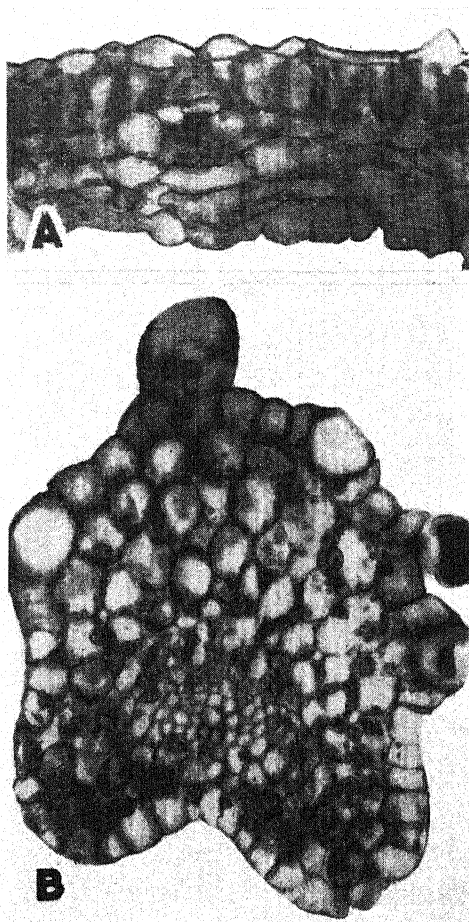


FIG. 4.—Section through (A) lamina of leaflet of plant treated with 2-chlorophenoxyacetic acid, illustrating failure to develop intercellular spaces; (B) tip of embryonic leaflet from plant treated with 2,4-D, illustrating maturity of tissue.

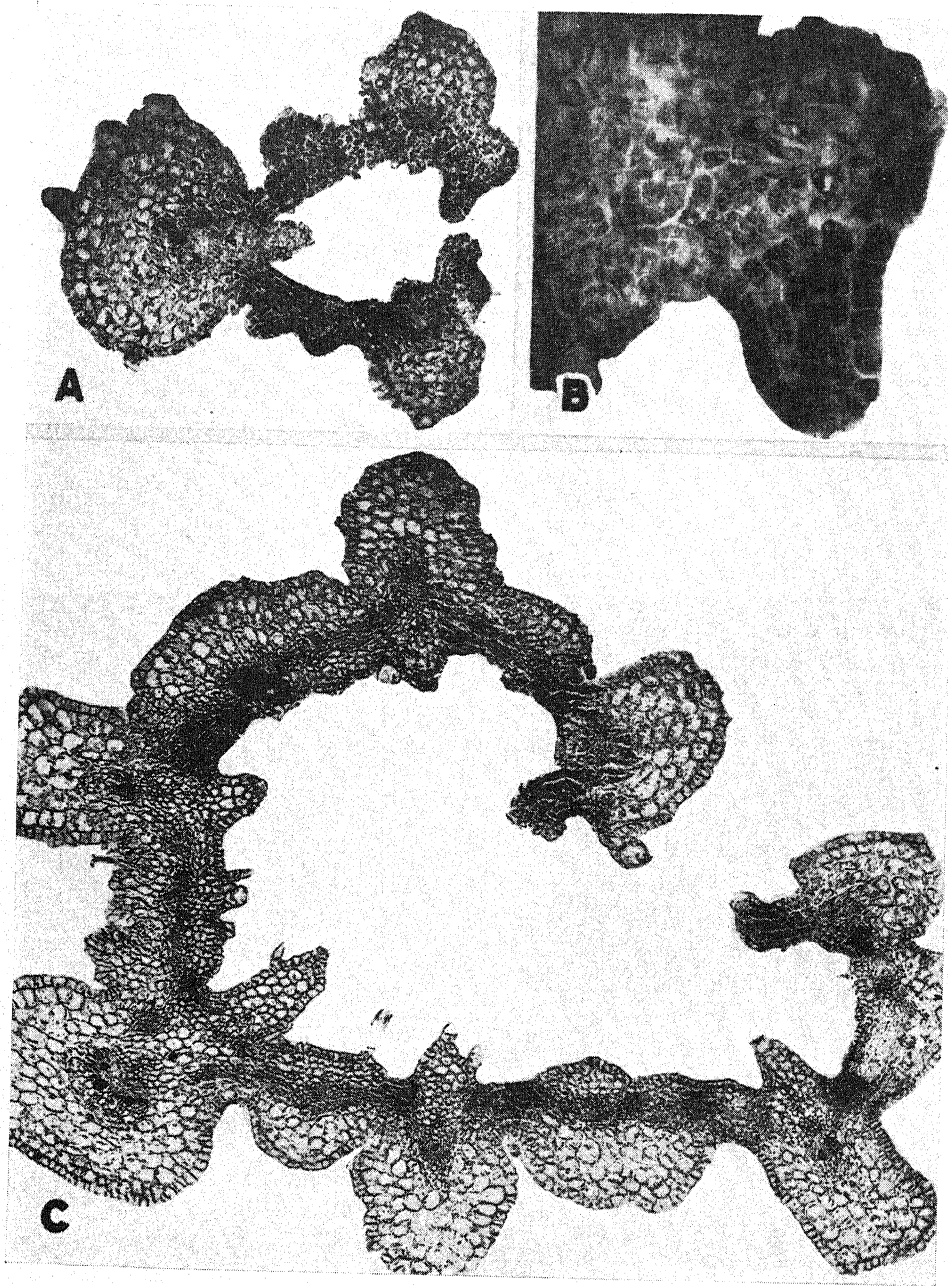


FIG. 5.—Cross sections of embryonic leaflet shown in fig. 4*B*, showing (*A*) origin of lamina and (*B*) details of marginal and plate meristems. *C*, cross section through extremely modified mature leaflet of plant treated with 2,4-D, showing fasciation of veins and chlorenchymatous tissue as dark-staining regions at margin and in islets between veins.

complete (fig. 6). Parenchyma surrounding the midrib and major veins was continuous from the upper to the lower epidermis. The parenchyma cells of the major veins contained no chlorophyll so that chlorenchymatous tissue was confined to a narrow margin of the leaflet. The vascular elements remained discrete and appeared as separate bundles imbedded in the parenchyma. Parenchyma cells farthest from the vascular elements were largest and those adjacent to the vascular elements were smallest. The vascular elements and the small parenchyma cells surrounding them formed a

plate within the larger plate of parenchyma. At the extreme margin were a few chlorenchymatous cells that usually were confined to the layer corresponding to palisade in normal leaves.

Discussion

The gross responses observed in treated bean plants were similar to those previously reported. SWANSON (9) sprayed Red Kidney bean plants with 2,4-D and noticed epinasty and stem curvature in a few hours. The plants he observed also produced tiny leaves. BEAL (2, 3, 4) treated sweet pea, African mari-

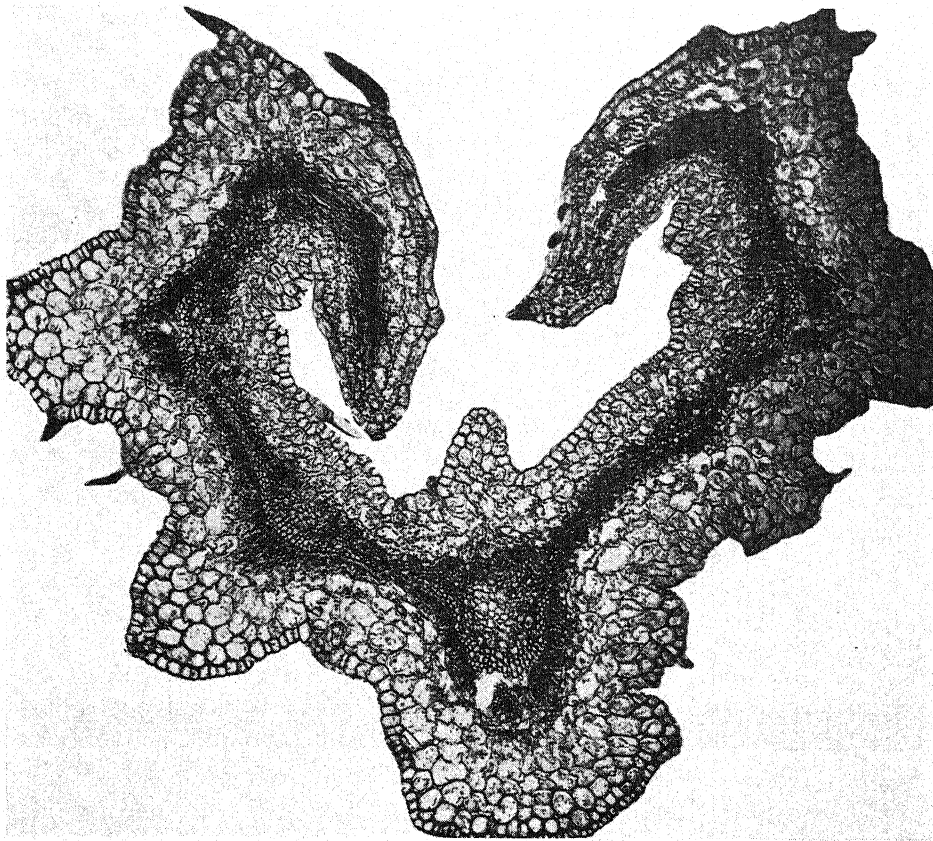


FIG. 6.—Cross section through narrow, rolled leaflet ultimately produced by plants treated with 4-chlorophenoxyacetic acid. Veins are fasciated, and vascular elements and small parenchyma cells surrounding them appear as darkly stained region across larger parenchyma region. Chlorenchyma confined to margin.

gold, and Red Kidney bean plants with substituted chlorophenoxy and other compounds. His experiments demonstrated that responses to treatment with 2-chlorophenoxyacetic acid applied in Carbowax were greater than when applied in lanolin. Conversely, 4-chlorophenoxyacetic acid applied in lanolin produced a marked response, although the same compound applied in Carbowax was ineffective. As in the present experiments, responses to 2,4-D were nearly indifferent to the carrier used.

MARTH and DAVIS (6) showed that temperature is of significance in the induction of responses to 2,4-D. If temperatures were too high, killing commonly resulted. In the present experiments concentrations of 0.5% of acid in the carrier were found to induce changes in the growth pattern at ordinary spring temperatures in a greenhouse. This concentration of 2,4-D and of 4-chlorophenoxyacetic acid was lethal at higher temperatures.

ZIMMERMAN and HITCHCOCK (12) reported formative effects of several phenoxy compounds on tobacco, tomato, and other plants. Some of their figures indicate progressive modification of leaves of plants treated with 4-chlorophenoxyacetic acid. The comparative stage of development of these leaves at the time of treatment is not clear. In the present study and in the experiments of BEAL (3), if the first trifoliate leaf was starting to emerge from the bud at the time of treatment with 4-chlorophenoxyacetic acid, its mature leaflets resembled the modified leaflets of plants treated with 2-chlorophenoxyacetic acid. Second and third trifoliate leaves, although usually similar in appearance, were much smaller. Subsequent leaves were almost uniform in size and had a narrow straplike form

(fig. 1E). These later leaves were present only as primordia or perhaps were not yet differentiated at the time of treatment. This suggests that, if leaves are young enough, a uniform response to treatment with 4-chlorophenoxyacetic acid might occur. There was a suggestion of eventual uniformity of response in ZIMMERMAN and HITCHCOCK's report of successive whorls of fasciated leaves of tobacco treated with 4-chlorophenoxyacetic acid.

Modification in response to applications of 2,4-D produced a gradation in size that extended to leaves not yet apparent in the buds at the time of treatment. On the basis of the present experiments, it could not be determined whether the reduction in size of leaves developed following the application of 2-chlorophenoxyacetic acid was similarly progressive or whether the size gradation was a consequence of the relative immaturity of the later-formed leaves at the end of the experiments.

The development of the lamina of the untreated Red Kidney bean leaflet is in many respects similar to that observed in tobacco by AVERY (1). The subepidermal, marginal meristem in both plants gives rise to four layers within the epidermis. These layers remain four in the bean but become five in tobacco by division of one of the two inner layers. In both plants the adaxial of these four internal layers become palisade parenchyma, while the other three internal layers (four in tobacco) differentiate into spongy mesophyll. Subsequent to the formation of the four layers of plate meristems in bean leaflets and the five layers in tobacco leaves, all cell divisions produced new cells in the plane of the plate meristems, except those involving vein formation. The divisions in this

plane led SCHUEPP (7) to term these layers "Plattenmeristem." SMITH (8) showed that the constancy in the number of layers, and hence extension of the lamina by growth of plate meristem, is of regular occurrence in embryonic leaves with the exception of certain sun leaves.

Veins of the minor vascular network in bean leaflets originate in the second layer beneath the adaxial epidermis. AVERY (1) stated that this is the usual mode of origin in tobacco. FOSTER (5), in his summary, showed diagrams indicating that veins usually originate in the layer immediately beneath the embryonic palisade or palisades.

According to AVERY (1), the development of intercellular spaces in mesophyll is a result of continued cell enlargement in the epidermis after cell division and enlargement in the mesophyll have ceased. The compounds used in the present experiments inhibited the formation of intercellular spaces. This possibly indicated that the epidermal cells of the laminae of bean plants treated with substituted phenoxy compounds failed to enlarge. There is also the possibility that lysis of the middle lamellae of mesophyll cells failed to occur. This would render epidermal cell enlargement ineffective in producing intercellular spaces. Since the mesophyll cells then would not separate, the epidermal cells would be forced to increase their volume by periclinal expansion.

The narrow leaflets of plants treated with 4-chlorophenoxyacetic acid indicated suppression of the activity of the plate meristem. The resulting approximation of veins resulted in fasciation. The parenchyma surrounding the veins became continuous although the vascular elements remained discrete. The laminae of leaflets from plants treated with 2,4-D

exhibited an internal structure in the earlier leaves similar to that induced by 2-chlorophenoxyacetic acid. The gradation from this form to leaflets resembling those from plants treated with 4-chlorophenoxyacetic acid has been noted. This indicated a progressive inhibition of cell division in the plate meristem, for the laminal meristem was present and functional.

Summary

1. A bean leaflet develops a lamina by the activity of a subepidermal marginal meristem, which produces four internal layers of plate meristem. The adaxial of these layers develops into the palisade layer; the other three produce the spongy mesophyll.

2. Veins of the minor network are initiated by divisions of a row of cells in the layer beneath the embryonic palisade. Additional cells and additional layers become involved.

3. Treatment of young plants with 0.5% concentration of 2-chlorophenoxyacetic acid in Carbowax inhibited the formation of intercellular spaces in the mesophyll of bean leaflets.

4. Similar application of 0.5% 4-chlorophenoxyacetic acid in lanolin inhibited the activity of plate meristem in the laminae of bean leaflets. As a result, veins were approximate and their parenchyma became continuous although the vascular elements remained discrete. Chlorenchymatous tissue was confined to the margin.

5. Similar treatment with 0.5% concentrations of 2,4-dichlorophenoxyacetic acid, in either lanolin or Carbowax as the carrier, induced progressive modification of bean leaves. The earliest leaves were similar to those induced by treatment with 2-chlorophenoxyacetic acid. In later

leaves the external form and internal structure resembled that found in leaflets of plants treated with 4-chlorophenoxyacetic acid.

The writer wishes to express his appreciation of the advice and encouragement

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COMPARATIVE HERBICIDAL VALUE OF 2,4-DICHLOROPHENOXYACETIC ACID AND 2,4,5-TRICHLOROPHENOXYACETIC ACID ON SOME HERBACEOUS WEEDS, SHRUBS, AND TREES UNDER HAWAIIAN CONDITIONS¹

R. K. TAM

Introduction

The use of 2,4-dichlorophenoxyacetic acid (2,4-D) as a herbicide has been emphasized in nearly all investigations since the early reports of MARTH and MITCHELL (7) and HAMNER and TUKEY (3), although the possible use of 2,4,5-

trichlorophenoxyacetic acid (2,4,5-T) for similar purposes was recognized by HAMNER and TUKEY (2). With the kidney bean and soybean plants, SWANSON (8) showed that 2,4,5-T and most of its derivatives, carried in oil, produced a greater inhibitory effect, in general, than 2,4-D. Another instance of the greater toxicity of 2,4,5-T and of other compounds with the 2,4,5-trichlorophenoxy-configuration was observed by ENNIS *et*

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al. (1) when tested on the Irish potato in both water and oil solutions.

In the last 2 years both 2,4-D and 2,4,5-T have been sprayed on many herbaceous weeds, shrubs, and trees growing under warm humid Hawaiian conditions. The investigations of VAN OVERBEEK and VELEZ (9) and of VAN OVERBEEK *et al.* (10) with 2,4-D under somewhat similar conditions are recognized. It is the purpose of this report to indicate the responses of some of these plants to 2,4-D and to point out several species of plants in which better kills resulted with 2,4,5-T.

Material and methods

Preliminary tests confirmed the observations of HAMNER and TUKEY (4) that, although solutions of 500 and 1000 p.p.m. of the compounds were acceptable for herbicidal use on some species of herbaceous weeds, higher concentrations (2000–5000 p.p.m.) were desirable for woody plants. For all the tests reported here, the acid forms of 2,4-D and 2,4,5-T were used, while conversion to the sodium and ammonium forms was often necessary to increase their solubility in water.

When aqueous solutions of 1000 p.p.m. or less were made up, Carbowax 1500 was used as the carrier. Solutions of 2000–10,000 p.p.m. were prepared by forming the sodium salt, using an equal amount of sodium bicarbonate; ammonium carbonate was used to form the ammonium salt. Wetting was enhanced by adding water-soluble Standard Wetting Agent (a sodium sulfonated aromatic prepared by the Standard Oil Company of California) at the rate of 2 lb./100 gal. dilute spray.

Diesel oil (Pacific Standard 200) solutions of 1000–20,000 p.p.m. (2.0%) of both 2,4-D and 2,4,5-T were prepared by

dissolving the acids in diethylene dioxide (1,4 Dioxane-practical) and by adding diesel oil up to 80% of the final solution. 2,4-D for testing was supplied by the American Chemical Paint Company, the Dow Chemical Company, and E. J. KRAUS. 2,4,5-T was obtained from J. W. MITCHELL, the American Chemical Paint Company, and the Dow Chemical Company. Maximum solubilities of samples of the same chemical obtained from the different sources have proved not to be equal under all conditions. Each sample was used only when complete solubility provided the desired concentration. Whenever possible, solutions were made up and used the same day.

Under Hawaiian conditions, a daily range in temperature from a minimum of 70° F. to a maximum of 85° F. occurs during a large percentage of the days in the year. There are few days in which temperatures range from 60° to 70° F., and this range is usually confined to locations of higher altitude. Prolonged periods of this range are rare. Suitable temperatures for the activity of the chemicals used are usually encountered the year round, being above the 50°–60° F. range in which retardation of kill has been shown to occur (6).

Rainfall under these subtropical conditions is a greater problem in the use of the herbicides. Heavy dew and some rainfall (especially in the upland areas) prevail for many nights and days from October to April. It was noted in several early tests that response to treatment was limited when light showers fell 6–24 hours after application. In one instance total lack of response occurred when a moderate rain fell within 2 hours after applying the chemicals. A prolonged period of high humidity for several weeks after treatment with 2,4-D and 2,4,5-T has resulted, in one case, in heavy forma-

tion of aerial roots on stems of several species of mature weeds. Despite severe twisting and swelling of stems, complete kill under these conditions was not obtained. Spraying of 2,4-D or 2,4,5-T in oil under these moist conditions, as suggested by WEAVER *et al.* (11), may render their action more effective.

Results

HERBACEOUS AND SEMIWOODY WEEDS.
—Weeds growing under natural conditions in the field were sprayed with 1000

p.p.m. of 2,4-D and the same concentration of 2,4,5-T. Volume of spray applied was 160 gal./acre. Weather following spraying was dry for at least 24 hours, and during the period of study the mean daily temperature averaged above 70° F. Total damage, as estimated from desiccation and defoliation of several plants, was reported as mean percentage kill (table 1).

Groups A and B in table 1 include a large percentage of the total population of common herbaceous and semiwoody

TABLE 1
PERCENTAGE KILL OF HERBACEOUS AND SEMIWOODY WEEDS, FOUND IN HAWAIIAN PINEAPPLE FIELDS, 6 WEEKS AFTER SPRAYING WITH AQUEOUS SOLUTIONS (1000 P.P.M.) OF 2,4-D AND 2,4,5-T

Species	2, 4-D	2, 4, 5-T
A. <i>Species equally affected by 2,4-D and 2,4,5-T:</i>		
Balsam apple (<i>Momordica balsamina</i> L.)	100	100
Field bindweed (<i>Convolvulus arvensis</i> L.)	100*	100*
Kikania, cocklebur (<i>Xanthium pennsylvanicum</i> Wallr.)	100	100
Castor bean (<i>Ricinus communis</i> L.)	100	100
Hialoa (<i>Waltheria americana</i> L.)	100	100
Wild indigo (<i>Indigofera suffruticosa</i> Mill.)†	100	100
Nettle-leaved goosefoot (<i>Chenopodium murale</i> L.)	100	100
Asiatic pennywort (<i>Centella asiatica</i> [L.] Urban)	100	100
Spanish needles (<i>Bidens pilosa</i> L.)	100	100
Horseweed (<i>Erigeron albidus</i> [Willd.] Gray)	100	100
Purslane (<i>Portulaca oleracea</i> L.)	100	100
Richardsonia (<i>Richardsonia scabra</i> [L.] St. Hil.)	100	100
B. <i>Species more affected by 2,4-D than by 2,4,5-T:</i>		
Swine's-cress (<i>Senebiera didyma</i> Persoon)	100	75
Cheese weed (<i>Malva rotundifolia</i> L.)	95	25
False mallow (<i>Malvastrum coromandelianum</i> [L.] Garcke)	100	25
Ilima (<i>Sida fallax</i> Walp.)	100	50
False ragweed (<i>Franeria strigulosa</i> Rydb.)	95	50
Lamb's-quarters (<i>Chenopodium album</i> L.)	100	75
Red pualele, Flora's-paintbrush (<i>Emilia sonchifolia</i> DC.)	100	75
Nutgrass (<i>Cyperus rotundus</i> L.)	100*	95*
Honohono (<i>Commelina nudiflora</i> L.)	100	95
C. <i>Species more affected by 2,4,5-T than by 2,4-D:</i>		
Popolo (<i>Solanum nodiflorum</i> Jacq.)	35	100
Hilahila, sensitive plant (<i>Mimosa pudica</i> L.)	50*	75*
Wood sorrel (<i>Oxalis martiana</i> Zucc.)	50*	100*
Phyllanthus weed (<i>Phyllanthus niruri</i> L.)	25	95
Christmas berry (<i>Schinus terebinthifolius</i> Raddi)	50	100
D. <i>Species not killed by either 2,4-D or 2,4,5-T:</i>		
Jimson weed (<i>Datura stramonium</i> L.)	25	25
Ageratum (<i>Ageratum conyzoides</i> L.)	50	50

* May require two or more treatments to effect control, owing to formation of new shoots.

† Young plants (3-6 in. tall).

broad-leaved weeds found in pineapple fields and wayside places. For the control of species listed in these two groups, it does not seem advantageous to use 2,4,5-T, since 2,4-D proved either equally or more toxic (fig. 1). Following treatment of areas heavily infested with nut-

grass (*Cyperus rotundus*), new shoots appeared more rapidly and in greater number with 2,4,5-T than 2,4-D. In special tests it was found that repeated sprayings, preferably with a 5000-p.p.m. aqueous solution, were necessary for reducing the population of this weed in in-

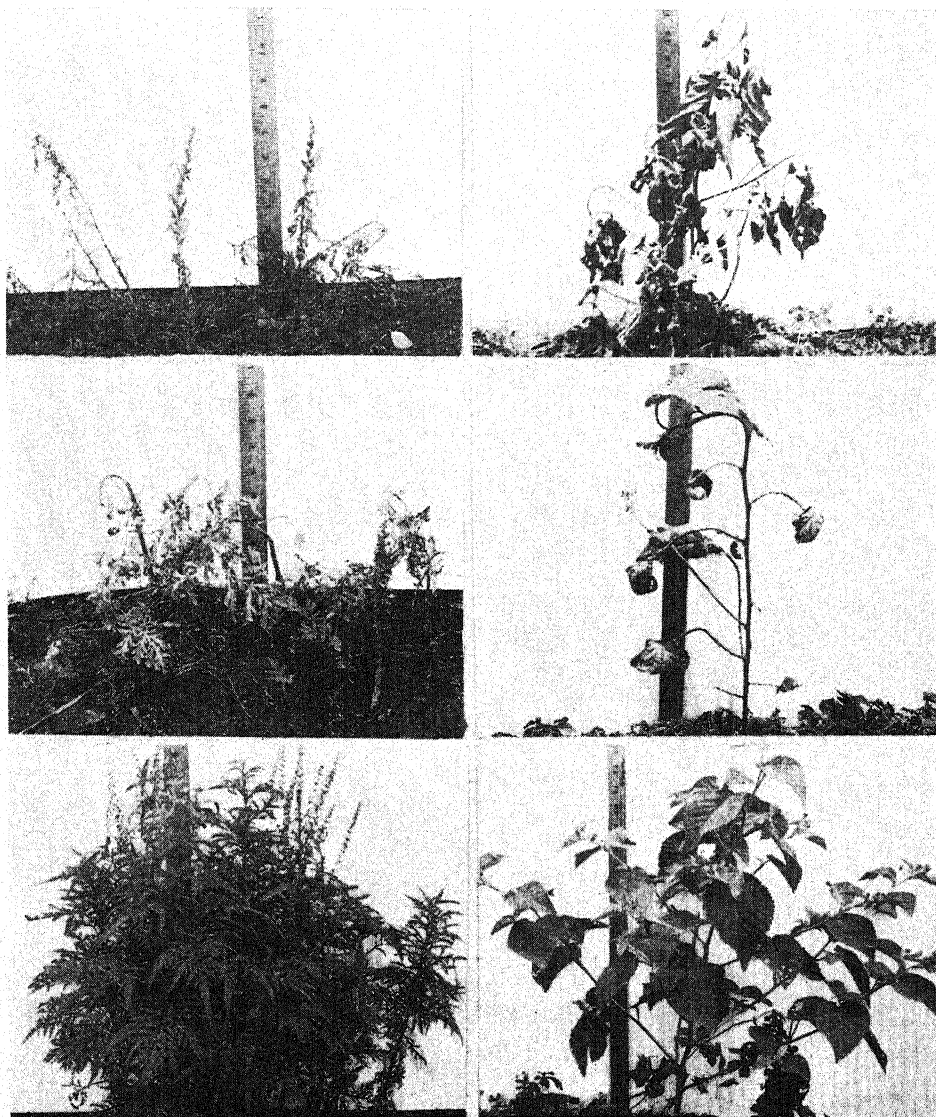


FIG. 1.—Greater toxicity of 2,4-D (*upper*) than of 2,4,5-T (*center*); applied as aqueous sprays (1000 p.p.m.) 6 weeks earlier. *Lower*, untreated. *Left*, *Franseria strigulosa*; *right*, *Sida fallax*.

fested areas. Oil solutions of 2,4-D rapidly scorched plants, but new stands were quickly formed.

Group C contains five species of weeds which appeared to be more affected by 2,4,5-T than 2,4-D when plants were sprayed with these chemicals at a con-

centration of 1000 p.p.m. Of these, popolo (fig. 2) and Christmas berry were shown, in several subsequent tests using higher concentrations of the chemicals, to be killed by 2,4,5-T while being unusually resistant to the activity of 2,4-D. In this first test, the Christmas berry



FIG. 2.—Greater toxicity of 2,4,5-T (center) than of 2,4-D (upper); applied as aqueous sprays (1000 p.p.m.) 6 weeks earlier. Lower, untreated. Left, *Eucalyptus citriodora*; right, *Solanum nodiflorum*.

plants were seedlings about 6 inches tall; the high toxicity of 2,4,5-T to this species was also indicated in other tests with mature trees 10-15 feet tall in which one spraying often resulted in complete kill. Plants in group D were not killed by either 2,4-D or 2,4,5-T.

SHRUBS AND TREES.—There are several species of shrubs and trees which infest valuable pasture land, and the extermination of some of these is highly desirable. Among the most serious and widespread are guava and lantana. 2,4,5-T was more toxic than 2,4-D to all species of woody shrubs and trees tested, with the exception of guava (table 2). Lantana was not greatly affected by 1000-p.p.m. solutions of 2,4-D and 2,4,5-T, although 2,4,5-T rated higher than 2,4-D. This was confirmed in later tests using stronger concentrations. The resistance of popolo to 2,4-D, as reported in table 1, was also noted in this test. *Eucalyptus citriodora* (fig. 2), when young was killed readily by 1000 p.p.m. of 2,4,5-T, while the same concentration of 2,4-D proved comparatively innocuous. The striking selectivity was further demonstrated in mature trees, 40-50 feet tall, which had been completely girdled 3 feet above the ground and painted on the wounds with 15% solutions of the chemicals in Carbowax 1500. When 2,4,5-T was applied, complete kill resulted, with 100% desiccation of foliage in about 6 months. In 2,4-D treated trees and in girdled but untreated trees excessive gum exudation followed with no visible systemic injury to the trees even after 20 months. In the case of false koa, new growth following complete defoliation was rapid; this is fortunate, since this plant is considered valuable as forage. The algaroba or kiawe (fig. 3) responded to both compounds, with 2,4,5-T slightly more toxic.

A later test was installed to check the toxicity of 2,4,5-T, including other woody pests not previously tested. An area of about 1 acre was sprayed with an aqueous solution of 2000 p.p.m. of 2,4,5-T. The high toxicity of this compound to the shrubs and trees listed in table 3 was undoubtedly influenced partially by continued warm and dry weather during this test. With the exception of pluchea, complete desiccation of nearly all the plants in the test area was

TABLE 2

PERCENTAGE TOXICITY TO SOME SHRUBS AND TREES OF AQUEOUS SOLUTIONS (1000 P.P.M.) OF 2,4-D AND 2,4,5-T, 6 WEEKS AFTER SPRAYING

Species	2,4-D	2,4,5-T
Guava (<i>Psidium guajava</i> L.)	95*	90*
Lantana (<i>Lantana camara</i> L.)	25*	50*
False ironwood (<i>Casuarina equisetifolia</i> L.)	90*	100*
Eucalyptus (<i>Eucalyptus citriodora</i> Hooker)	25*	100
Algaroba, kiawe (<i>Prosopis chilensis</i> [Molina] Stuntz)	95	100
False koa (<i>Leucaena glauca</i> [L.] Benth.)	75*	100*
Popolo (<i>Solanum nodiflorum</i>)	25	100

* Requires two or more sprayings, since new shoots appeared 2-3 months after first application.

evidenced. Subsequent formation of new shoots in some isolated instances, in which coverage by spraying was possibly incomplete, would have necessitated only a limited amount of spot spraying for complete control.

Recovery was noted to be more consistent and more rapid in lantana. This species may require even more concentrated solutions of 2,4,5-T for complete control with a single application. Defoliation following treatment was 100% in this species; it was noted, in all tests conducted to date, that regeneration is most likely in those species in which defoliation takes place in response to treat-

ment. When 100% desiccation of leaves occurs with no noticeable defoliation for a long period following spraying, a complete kill of the large woody plants tested is practically a certainty. It was also noted that, in cases in which special care had been taken to wet all the stem surfaces above ground, chances of a complete kill were greater than if only the leaves were sprayed.

Results from the foregoing tests showed that, despite desiccation and complete defoliation with some species, notably lantana and guava, complete kill was often not obtained with one

spraying of the dilute chemicals. It was thought that a complete kill might be accomplished by a high concentration of the chemicals carried in oil. Subsequently, several species, including mostly large mature plants, were sprayed with 2.0% solutions of 2,4-D and 2,4,5-T (table 4). The selectivity of 2,4-D for guava and of 2,4,5-T for eucalyptus was not evident when both chemicals were used in the high concentration of 2.0% in diesel oil. Against lantana, which has proved to be one of the hardest plants to kill, 2,4,5-T again proved to be more toxic than 2,4-D, even in high concen-

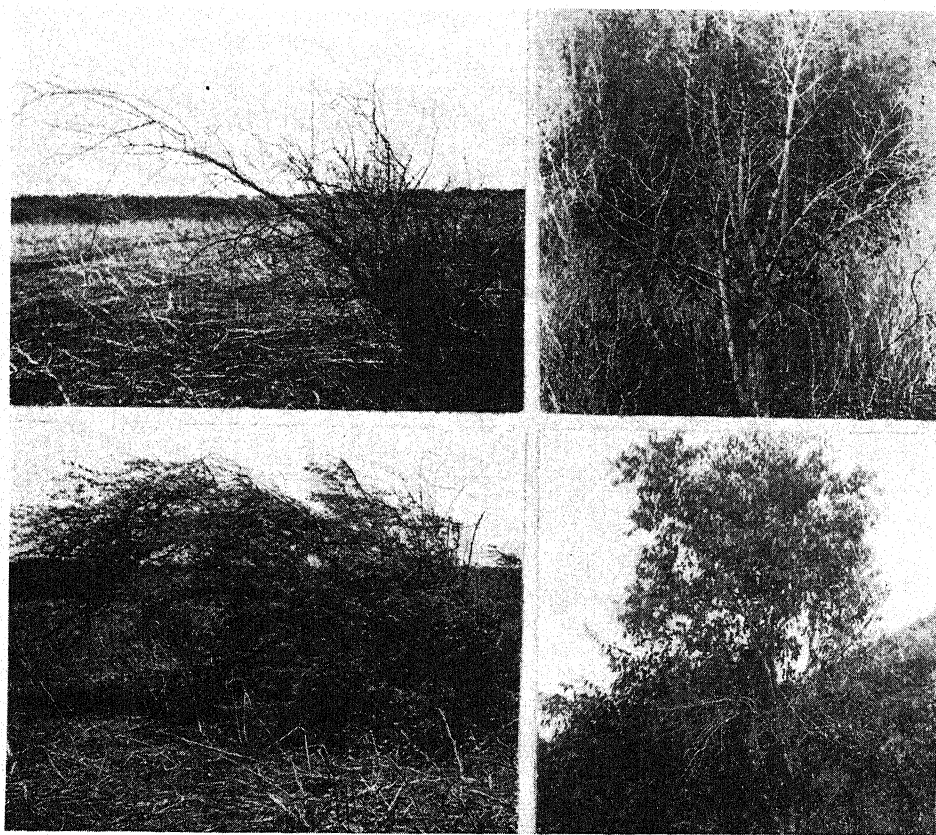


FIG. 3.—Toxicity of 2,4,5-T to (upper left) *Prosopis chilensis* and (upper right) *Eugenia jambolana*, applied as aqueous spray (2000 p.p.m.) 12 and 6 weeks earlier, respectively. Lower left, same *Prosopis* before spraying; lower right, untreated *Eugenia*.

trations. Use of these chemicals in high percentage and low volumes may find practical application on these and other resistant plants.

PINEAPPLES.—The possibility of using such herbicides for weed control in fields of growing pineapples was investigated (table 5). When sprayed at the rate of 160 gal./acre, concentrations of 2,4-D up to 10,000 p.p.m. did not completely kill 8-month-old pineapple plants. With both sodium and ammonium salts of 2,4-D, a maximum toxicity of 95% was indicated at the highest concentration. Formation of new ground suckers proved that, with some plants, portions of the stem below the soil surface were not killed. In contrast to the low toxicity of both sodium and ammonium 2,4-D at 2000 p.p.m., 2,4,5-T in the same concentration was at least 95% toxic. Use of solutions of 5000 and 10,000 p.p.m. of 2,4,5-T resulted in 100% kill. The inability of the plants treated with 2,4,5-T to form new ground suckers indicated

40% toxicity, as in the case of 2000 p.p.m. of 2,4-D, precludes the use of this type of weed-killer as a spray in pineapple fields.

Discussion

In tests which compared the effects of 2,4-D and 2,4,5-T on plants growing in a

TABLE 3

PERCENTAGE DESICCATION AND DEFOLIATION OF SHRUBS AND TREES 6 WEEKS AFTER SPRAYING WITH 2,4,5-T (2000 P.P.M.)

Species	Desiccation	Defoliation
Christmas berry (<i>Schinus terebinthifolius</i>).....	100*	0
Java plum (<i>Eugenia jambolana</i> Lamarck).....	100*	50
Lantana (<i>Lantana camara</i>).....	100*	100
Pluchea (<i>Pluchea odorata</i> [L.] Cass.)	50*	50
Castor bean (<i>Ricinus communis</i>)...	100	75
Cat's-claw (<i>Caesalpinia sepiaria</i> Roxb.).....	100*	100
Eucalyptus (<i>Eucalyptus citriodora</i>)	100	0
False ironwood (<i>Casuarina equisetifolia</i>).....	100*	0

* May require two or more sprayings, since in some cases with large plants new shoots appeared 2 or 3 months after first application.

TABLE 4

PERCENTAGE KILL OF SPECIES OF SHRUBS AND TREES 12 WEEKS AFTER APPLICATION OF 2.0% 2,4-D AND 2,4,5-T IN 20% DIOXANE AND 80% DIESEL OIL

Species	2,4-D	2,4,5-T	Diesel oil control
Guava (<i>Psidium guajava</i>).....	100	100	25*
Lantana (<i>Lantana camara</i>).....	95*	100	25*
Eucalyptus (<i>Eucalyptus citriodora</i>).....	100	100	10*
False ironwood (<i>Casuarina equisetifolia</i>).....	100	100	75*
Wild indigo (<i>Indigofera suffruticosa</i>)†.....	100	100

* New shoots appeared.

† Mature plants 3-4 ft. tall.

that not only were the parts of the plant above the ground destroyed but also that the portions of stem below the ground were rendered incapable of regeneration. On the basis of these results, 2,4,5-T in low concentration appeared to be approximately twice as toxic to pineapples as 2,4-D. However, even a

semiotropical region, results indicated that with some species better kills were obtained with 2,4,5-T than with 2,4-D. These were, in general, plants classified as shrubs and trees. With the more susceptible, small, common herbaceous weeds of lawn and field, 2,4-D was either more effective, or, as in most cases, little

difference was found between it and 2,4,5-T.

Eucalyptus citriodora is an outstanding example of a species to which 2,4,5-T is more toxic than 2,4-D. When it was sprayed with aqueous solutions at 1000-2000 p.p.m., it was little damaged by 2,4-D, whereas 2,4,5-T gave complete kills with only one spraying on plants 5-10 feet tall. This differential toxicity was further demonstrated when trees 40-50 feet tall were girdled and concen-

especially on the woody types which are hard to kill, would furnish valuable information on its possible use as a supplement to 2,4-D in a general weed-control program. The use of 2,4,5-T in oil seems advantageous, since high concentration is possible, and, coupled with high or low pressures and special nozzles (5), atomization makes it possible to spray large trees with small volumes per acre. The greater inhibitory effect per unit of 2,4,5-T or its derivatives (8) in oil, plus

TABLE 5

TOXICITY TO 8-MONTH-OLD PINEAPPLE PLANTS OF SODIUM 2,4-D, AMMONIUM 2,4-D, AND AMMONIUM 2,4,5-T IN AQUEOUS SOLUTION, SPRAYED AT RATE OF 160 GAL./ACRE. READINGS 3 MONTHS AFTER APPLICATION

Treatment (no.)	Chemical	Concentration (p.p.m.)	Toxicity (%)	Ground suckers (no.)
1.....	Sodium 2,4-D	2,000	40
2.....	Sodium 2,4-D	5,000	75
3.....	Sodium 2,4-D	10,000	95	2
4.....	Ammonium 2,4-D	2,000	55
5.....	Ammonium 2,4-D	5,000	75
6.....	Ammonium 2,4-D	10,000	95	5
7.....	Ammonium 2,4,5-T	2,000	95	0
8.....	Ammonium 2,4,5-T	5,000	100	0
9.....	Ammonium 2,4,5-T	10,000	100	0

* Mean of forty plants in each treatment.

trated Carbowax solutions of the two compounds were applied to the cut area. 2,4,5-T treatment resulted in complete kill in 6 months, while plants girdled and untreated or treated with 2,4-D were alive after 20 months. Only when plants were completely covered with 2.0% 2,4-D and 2,4,5-T sprays in diesel oil was the selectivity of 2,4,5-T for eucalyptus not in evidence; under these conditions both 2,4-D and 2,4,5-T produced 100% kills.

Although the herbicidal value of 2,4-D for most herbaceous weeds has been extensively investigated and confirmed, it seems that future trials with 2,4,5-T,

the possible minimizing of the deleterious effects of rainfall by this method (11), should prove valuable under most conditions and especially under the moist subtropical Hawaiian conditions.

Summary

1. Some species of herbaceous and semiwoody weeds, shrubs, and trees were sprayed with 1000-2000 p.p.m. aqueous solutions of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) under warm humid Hawaiian conditions. In addition, several woody species were sprayed with

2.0% concentrations of the chemicals in diesel oil.

2. When sprayed on species of herbaceous or semiwoody weeds, 2,4-D was either greater than, or equal to, 2,4,5-T in toxicity in aqueous solution at 1000 p.p.m.

3. When tested on some species of shrubs and trees, 2,4,5-T was observed to be more toxic than 2,4-D. Woody species, such as eucalyptus (*Eucalyptus citriodora*), popolo (*Solanum nodiflorum*), sensitive plant (*Mimosa pudica*), Christmas berry (*Schinus terebinthifolius*), lantana (*Lantana camara*), false ironwood (*Casuarina equisetifolia*), algaroba (*Prosopis chilensis*), Java plum (*Eugenia jambolana*), and cat's-claw (*Caesalpinia sepiaria*) were more injured by 2,4,5-T treatment. The guava (*Psidium guajava*) was an exception, being damaged more by 2,4-D than by 2,4,5-T.

4. Eucalyptus was killed with one spraying of 1000 p.p.m. of 2,4,5-T, while the same concentration of 2,4-D did little damage. The specificity of 2,4,5-T for this species was further demonstrated with mature trees, 40-50 feet tall, when

girdled trees were killed in 6 months after 2,4,5-T was applied to the wounds, while plants treated with 2,4-D were alive 20 months after treatment.

5. With the exception of lantana, which proved to be one of the most difficult species to control, 2.0% solutions of 2,4-D and 2,4,5-T in diesel oil proved to be equal in toxicity to several woody species tested. Although the specificity of 2,4,5-T for eucalyptus was not in evidence at this high concentration, lantana was destroyed with one spraying of 2,4,5-T while 2,4-D-treated plants required repeated sprayings for control.

6. When sprayed on 8-month-old, actively growing pineapple plants, ammonium 2,4,5-T in aqueous solution at 2000-10,000 p.p.m. proved practically 100% toxic, while similar solutions of 2,4-D possessed much lower toxicity. In these tests 2,4,5-T was approximately twice as toxic as 2,4-D, but the damage produced by both compounds precludes their use as herbicidal sprays in fields of growing pineapples.

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STUDIES ON THE CAROTENE-DESTROYING PROCESSES IN DRYING BEAN LEAVES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 595

LEON BERNSTEIN AND JOHN F. THOMPSON

Introduction

It is generally recognized that field-curing of hay crops results in large losses of carotenoid pigments (1, 8, 12). These losses have been variously ascribed to the action of an enzyme and to light, different investigators emphasizing one or the other mechanism. HAUGE and co-workers (5, 6, 9, 10) have maintained that enzymic activity is mainly responsible for carotene loss, while GUILBERT (4) has emphasized the importance of light.

The terms "enzymic destruction" and "photodestruction" of carotene will be used in this paper. It is realized that if the enzyme involved in this process is a lipoxidase, as MITCHELL and HAUGE believe (9), then there is a coupled oxidation involving fats (14, 16) and not a direct enzymic oxidation with carotene as the substrate. It will be shown that photodestruction is likewise indirect, involving at least the absorption of light by substances other than the carotenoids. For brevity, however, the indirect nature of the processes will not be referred to again in naming them.

MITCHELL and HAUGE (10) measured the carotene loss in alfalfa drying in the light and in the dark in order to determine the relative importance of enzymic destruction and light-activated destruction. Although they had to assume that enzymic destruction of carotene was the same in the light and in the dark, they realized the weakness in their assumption and enumerated factors which

would cause differences in enzymic destruction between light and dark. It is impossible, therefore, to reach any conclusion as to the amount of carotene destroyed by light.

GUILBERT (4) compared the loss of carotene in sun-curing of autoclaved and unautoclaved alfalfa and concluded that photodestruction was responsible for a large fraction of the loss. MITCHELL and HAUGE claimed (10), however, that autoclaving affects the stability of carotenoids to subsequent exposure to light, so that it is not valid to compare carotene losses in autoclaved and unautoclaved leaves in the light as a means of determining the photo-oxidative loss. A further objection is that photodestruction is effective in autoclaved leaves from the moment of exposure to light, since the cells are already killed, whereas in fresh leaves a period must elapse during which the cells are killed or injured by partial drying before photodestruction (and enzymic destruction) becomes operative (see below).

With the restricted validity of the above-mentioned experimental techniques in mind, the present work was undertaken. Duplication of field conditions was sacrificed in favor of controlled environments, so that for specified conditions, at least, the extent of photodestruction and enzymic destruction could be quantitatively determined. The effects of varying environmental and internal factors—including the time factor, temperature, oxygen tension, light in-

tensity, light quality, water content of the leaf, and the presence or absence of naturally occurring, water-soluble antioxidants—on the rates of photodestruction and/or enzymic destruction have been investigated.

Material and methods

The leaves of Red Kidney bean plants were chosen as the experimental material because of their ready availability and because of the ease in preparing uniform samples, especially when orientation of the leaves to light is an important factor. Cordate leaves of plants grown in greenhouse soil to the stage when these leaves are fully expanded were used. The half-leaf method was employed. By cutting out the midrib, two sets of matched half-leaves were obtained; one was used as a control, the other as a treatment. Ten leaves sufficed to give matched sets which agreed within the limit of the chromatographic technique employed for carotenoid determinations. Analysis of six pairs of sets (ten half-leaves per set) gave a mean difference between matched sets of 1.0 ± 0.3 microgram of carotene per gram of fresh weight (less than 1% of the carotene content), with a maximum difference of $2.1 \mu\text{g/gm.}$ (1.7%).

The chromatographic method employed was essentially that of ZSCHEILE and WHITMORE (19) for fresh plant material. Modifications of the method included complete transfer of pigments from the aqueous phase to Skellysolve following extraction and the use of calcium hydroxide (approximately 1–2 cm. thick) to overlay the magnesia in the column. The calcium hydroxide gave better retention of the chlorophylls and allowed for elution and measurement of carotenols by 40% acetone in Skellysolve, following the elution of carotenes, without contamination by chlorophylls.

The term "carotenol" as used in this paper is equivalent to the more generally used term "xanthophyll." The carotenol fraction consisted of approximately 80% luteol, the remainder including two or more other carotenol pigments. Carotenol values were calculated from the standard carotene curve and are of significance for comparative purposes only.

The criterion for carotene destruction was decolorization of carotene as measured at 440 m μ according to the method of ZSCHEILE and WHITMORE.

Treatment of leaves by freezing, autoclaving, or drying prior to exposure to experimental conditions was necessary to insure controlled initiation of carotene destruction at the start of the experiment.

Our observations indicate that at least intensive injury, if not death, of cells is necessary for any marked carotene destruction. Conditions such as freezing or autoclaving which kill leaves, or a degree of dehydration sufficient to cause injury, are necessary pretreatments for rapid enzymic destruction and/or photodestruction of carotene. It has been postulated that the change in cells upon death which accounts for the increased susceptibility of carotene to enzymic destruction is due to increased contact between enzyme and lipoidal constituents resulting from disorganization of cell structure (13, p. 117).

Of the treatments employed, predrying has the advantage of being more comparable with field-curing conditions than either freezing or autoclaving. Freezing has an advantage over predrying, however, because there is no loss of carotene in pretreatment and cells are uniformly killed, resulting in more or less equal enzymic activity throughout the leaf. Preautoclaving has an advantage over predrying in that the cells are

uniformly killed and the enzymes inactivated.

Leaves were predried at room temperature ($22^{\circ}\text{C}.$) in a vacuum oven containing a generous supply of anhydrous calcium chloride, so that complete dryness could be obtained in $1\frac{1}{2}$ –2 hours. In this manner, carotene loss before the leaves had reached a given moisture content was kept small (5% of original carotene content), and destruction of carotene could be initiated at a precise time. Prefreezing was accomplished by cooling the leaves to $-18^{\circ}\text{C}.$ for 16 hours. Autoclaved leaves were heated at 5 pounds pressure in an autoclave ($108^{\circ}\text{C}.$) for 5 minutes.

In the studies on photodestruction the leaves were irradiated by a mercury vapor lamp (400-watt, type H-1, G.E.) equipped with a water filter. The incident light intensity usually employed was between 1000 and 1500 foot-candles. In the studies on light quality a 1000-watt tungsten-filament lamp was substituted as the light source. The leaves either were floated on water to prevent drying during exposure or more usually were dipped in heavy, white mineral oil and spread out in an open dish. The oil had no effect on rates of enzymic destruction or photodestruction as determined by comparison with leaves in moist-chambers. Exposure of leaves not covered by mineral oil in moist-chambers resulted in large losses of water from the leaves owing to slight heating of the irradiated leaves above air temperature. Leaves dipped in mineral oil in open dishes lost water equivalent to less than 10% of their fresh weight during 6 hours of exposure to 1300 foot-candles. At the end of the experiment most of the oil was removed by rinsing the leaves lightly in Skellysolve B. Control sets (kept in the dark during the experimental period) received the same mineral-oil treatment.

In experiments with predried or pre-frozen leaves, dark controls were used to correct for the enzymic destruction which occurred in sets exposed to light. With autoclaved material, control sets in the dark served to correct for a non-enzymic oxidation of carotene which, under the conditions of time and temperature employed, was in most cases negligible. Loss in carotenoid pigment is expressed either on an absolute basis or as a percentage of initial carotene content (as determined in control sets of autoclaved samples, or in separate samples, where controls were used to determine enzymic destruction).

All samples which were not autoclaved prior to experimental exposure were blanched in boiling water for 2 minutes at the conclusion of the run. All samples were stored at $-18^{\circ}\text{C}.$ until analyzed.

Experimentation

PRELIMINARY EXPERIMENT.—In one of our first attempts to evaluate the relative importance of enzymic destruction and photodestruction of carotenoids, fresh and autoclaved leaves were exposed to sunlight (6000 foot-candles), while comparable samples were kept near by in the dark (same air temperature). The exposed sets (light and dark) were taken up at intervals and re-weighed to determine moisture loss. Carotene loss in exposed sets was calculated as a percentage of initial carotene content.

In table 1 data are grouped together according to the amount of water lost, and the mean carotene loss for each group is reported. Fresh leaves lost approximately 30% of their carotene in the dark, while autoclaved leaves lost only about 1%, indicating that an enzyme-mediated destruction of carotene exists. This conclusion is based on the assumption that the only effect of autoclaving

pertaining to carotene destruction is in inactivating enzymes, although other explanations have been advanced (13, p. 116; 18). Autoclaved leaves in the light lost 53% of their carotene in 2-3 hours, while comparable leaves in the dark lost only about 1% up to 29 hours. This proves that a light-activated destruction of carotene can occur.

In fresh leaves in the light, both the enzyme and the light-activated mechanisms are responsible for the destruc-

ficed in favor of precisely controlled internal and external factors.

TIME-RATE STUDIES.—Knowledge of the influence of time on the rate of destruction of carotene is essential for the study of other factors affecting the process. A preliminary experiment showed that maximum enzymic destruction of carotene occurred when leaves had lost 50-90% of their water content. In another experiment leaves were dried at room temperature until 85-91% of the

TABLE 1
CAROTENE LOSSES IN DRYING LEAVES IN LIGHT (6000 FOOT-CANDLES) AND IN DARK

Leaf condition	Illumination	Weight loss as percentage of original fresh weight	Leaf temperature (°C.)	Time of treatment	No. of samples	Percentage carotene loss \pm standard error
Fresh.....	Light	50-62	40-42	20-60 min.	6	13.1 \pm 2.45
Fresh.....	Light	70-78	40-42	45-180 min.	5	35.6 \pm 2.20
Fresh.....	Light	80-90	40-42	60-130 min.	3	51.9 \pm 4.20
Autoclaved.....	Light	70-75	40-42	50 min.	2	34.0 \pm 3.95
Autoclaved.....	Light	84-88	40-42	60-170 min.	5	53.3 \pm 2.74
Fresh.....	Dark	20-30	30	30-65 min.	4	4.08 \pm 0.83
Fresh.....	Dark	40-60	30	120-180 min.	4	10.2 \pm 1.68
Fresh.....	Dark	80-90	30	18-29 hr.	5	31.0 \pm 1.42
Autoclaved.....	Dark	84-90	30	2 $\frac{1}{4}$ -29 hr.	5	1.06 \pm 0.58

tion of carotene during drying, but it is impossible from these data to calculate what part of total destruction was due to each mechanism. Fresh leaves in the dark cannot be used in measuring the extent of enzymic destruction which occurred in the light, because differences in leaf temperature and moisture content at any given time could have caused the amount of enzymic destruction in the light to differ from that in the dark. For reasons already mentioned, autoclaved leaves in the light cannot serve in measuring photodestruction in fresh leaves in the light. In an attempt to obviate these difficulties, simulated field conditions (gradual drying of material) were sacri-

moisture had been evaporated and then incubated at 37° C. for various periods of time, allowing no further change in moisture content. Control samples were blanched after the dehydration process. Curve *B* (fig. 1) presents these data, which show a large initial rate of carotene loss and subsequent decrease in rate. This results in leveling-off of the carotene loss curve in the region in which 40-50% of the carotene has been destroyed. The change in rate of carotene loss necessitates using an incubation period of 2 hours or less to measure the enzymic rate of destruction. At the present time we have no evidence as to the cause for the virtual cessation of enzymic destruction

after 40–50% of the carotene has been destroyed. This phenomenon has been observed (13, pp. 116, 118) with barley leaves but apparently does not occur in alfalfa (10). Curve A (fig. 1) shows how the carotene destruction in prefrozen leaves (incubated at 37° C.) varies with time. The curve is very similar to that for dehydrated leaves; this is construed to mean that enzyme action in prefrozen and in partially dehydrated leaves is the same. The fact that the curve for dehydrated leaves lies below that for prefrozen ones is probably due to lower en-

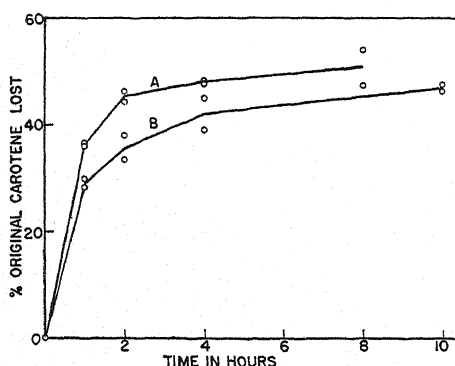


FIG. 1.—Effect of incubation period on amount of enzymic destruction of carotene. A, prefrozen leaves; B, predried leaves (85–91% of water removed). Incubation temperature, 37° C.

zymic activity in marginal cells which are too dry for optimal enzymic activity and in cells along veins where water content is too high for initiation, or maximum rate, of enzymic activity (fig. 3).

The influence of the time factor on the rate of photodestruction of carotenoids was studied with partially dehydrated leaves (fig. 2) and autoclaved leaves (fig. 9). Matched sets of leaves were dipped in mineral oil after pretreating (vacuum drying or autoclaving), one set then being exposed to light of a mercury vapor lamp, the other set serving as a dark control. Leaves which are partially dried

(68–85% of water removed) before being exposed to light show an initially high rate of photodestruction and a decrease in rate of photodestruction at the point where further enzymic destruction practically ceases. After 24 hours these leaves still retained approximately 30 μ g. of carotene (28%) and almost 100 μ g. (55%) of carotenol per gram of original fresh weight. This incomplete destruction of carotenoids is probably due in part to incomplete killing of the leaf tissues. However, even in leaves completely killed by prefreezing, photodestruction of carotenoids is not carried to completion as rapidly as in autoclaved leaves. Figure 9 shows that, after only 6 hours, 80–90% of the carotene of autoclaved leaves is destroyed by the action of light alone. Autoclaving therefore renders the carotenoids susceptible to more complete photodestruction, a fact which has been observed by MITCHELL and HAUGE (10) and by JONES *et al.* (7). This effect makes comparison of carotene destruction in autoclaved and unautoclaved leaves in light invalid as a means of determining enzymic destruction by difference, since autoclaving does more than simply eliminate enzymic destruction of carotene. Thus GUILBERT's (4) work does not give a true estimate of the relative importance of enzymic and photodestruction of carotene.

From the data in figure 2, certain valid comparisons of the rates of photodestruction and enzymic destruction can be made. During the first 8 hours the leaves in the dark lost 28 μ g., while the leaves in the light lost 63 μ g. of carotene per gram of original fresh weight. This would mean 28 μ g. lost by enzymic destruction and 35 μ g. lost by photodestruction. This is a valid comparison only for the conditions of the experiment. If the leaves had been predried until 85–

90% of the water had been lost instead of only about 75%, then the enzymic destruction would have been higher and would have accounted for approximately 40% of the initial carotene content (fig. 1), and the photodestruction might have been somewhat less (fig. 4). Another factor which renders difficult the comparison of photodestruction and enzymic destruction is the fact that in bean leaves the rate of enzymic destruction falls off sharply after 40% of the carotene has been destroyed. Therefore, quite different results can be obtained by comparing enzymic and photodestruction after 2, 8, and 24 hours of exposure of the leaves. If the variability of light and temperature encountered in the field is considered, it becomes evident how difficult it would be to evaluate the importance of enzymic and photodestruction under field conditions. By following a procedure such as used in this work (predrying or prefreezing), however, it is possible to determine the effects of various factors on the rates of photodestruction and enzymic destruction. In other words, we have been able to separate and distinguish these processes at least under controlled conditions, so that further study of the individual processes is possible. Finally, the relative amounts of photodestruction and enzymic destruction observed in this and other experiments indicate strongly that under ordinary field conditions of sun-curing, both the enzyme- and the light-mediated destruction of carotenoids are of importance and must be considered in any program to improve retention of carotenoids in leaves.

MOISTURE CONTENT AND CAROTENE DESTRUCTION.—Having determined appropriate time intervals for the study of rates of carotene destruction, the influence of moisture content on the rate of enzymic carotene destruction was in-

vestigated. A number of leaf sets were dried *in vacuo* at room temperature to different moisture contents. Control sets were blanched at this point, and remaining samples were incubated in the dark for 1 hour at 37° C. The maximum enzymic destruction occurs when 80–95% of the water has been removed (fig. 3). Since the curve showing the rate of caro-

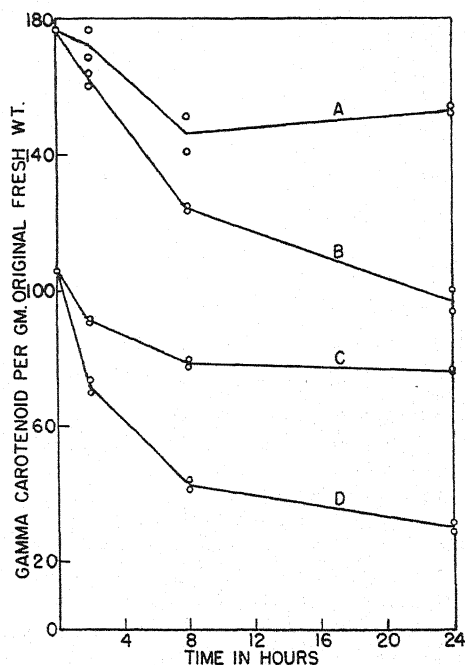


FIG. 2.—Carotenoid content of predried leaves (68–85% of water removed) incubated in dark and in light as a function of time. A, carotenol, dark; B, carotenol, light; C, carotene, dark; D, carotene, light. Light intensity, 1300 foot-candles; temperature, 23°–24° C.

tene destruction rises steeply when 75–82% of the water had been lost, death of the cell, or sufficient injury to cause accelerated carotene destruction, may be assumed to occur in this moisture range. That there are measurable carotene losses when 25% of the moisture had been removed is construed to mean that some of the marginal cells have lost 75%

or more of their moisture. The abrupt drop in rate of carotene destruction after 95% water loss means that the amount of water required for enzymic activity is very small. The fact that the rate of carotene destruction fell to zero when leaves were completely dry shows that some water is essential for enzymic activity. It should be pointed out that enzymic destruction of carotenoids is relatively little influenced by moisture content

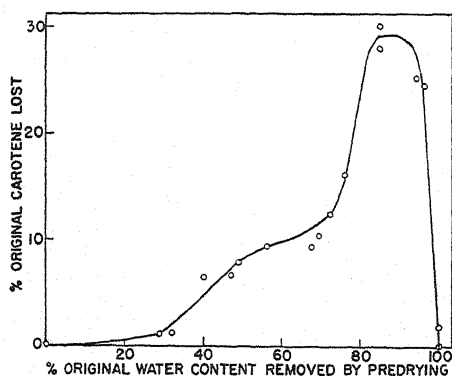


FIG. 3.—Effect of moisture content on enzymic destruction of carotene. Leaves predried *in vacuo* at room temperature before incubation at 37° C. Carotene loss calculated on basis of carotene remaining after dehydration. Incubation time, 1 hour.

(except in completely dry leaves) if the cells are prekilled by freezing.

The effect of the water content of bean leaves on photodestruction was studied with predried leaves prepared in the same way as described above. The leaves, after partial drying to desired water content, were dipped in mineral oil and spread out in open dishes. One set of each pair of matched sets was placed in the light for 3 hours, while controls were kept in the dark at the same temperature (24°–25° C.). The difference in carotene and carotenol content between dark controls and sets exposed to light is a measure of the amount of photodestruction which occurred (fig. 4). Replicate experiments were run in the morning and

afternoon of the same day. Although the general shape of the curves is the same, indicating maximum photodestruction in leaves which had lost approximately 75% of their water in predrying, the absolute values for the samples harvested and run in the afternoon are considerably higher than those for the morning. This indicates a marked effect of physiological condition of the leaf, influenced possibly by previous exposure to light, on the stability of carotenoid pigments to photodestruction under the conditions employed.

Maximum photodestruction occurs at a higher water content (at 65–85% dehydration; i.e., 65–85% of water removed in predrying) than maximum enzymic destruction (approximately 80–95% dehydration). This is due in part to the decrease in leaf area with drying and consequent decrease in light absorption per unit of pigment. However, photodestruction decreases to a greater extent than can be accounted for by shrinkage of the leaf as it approaches complete dryness. Leaves which were 89% dehydrated lost 33% less carotene through photodestruction than did leaves 75% dehydrated. The decrease in leaf area, however, was only 18%. The discrepancy is even larger when leaves 100% dehydrated are compared with those which were 89% dehydrated. Here the decrease in leaf area was 19%, while the amount of photodestruction decreased 71%. It is clear that water, directly or indirectly through effects on factors other than leaf area, increases the rate of photodestruction in partially dried leaves in the region of 75–100% dehydration. However, some photodestruction does occur in leaves which are completely dry. The maximum rate of photodestruction with reference to water content, therefore, lies between that water loss which is necessary to kill

or injure the cell and the point at which further loss of water retards the photodestructive process. Since all the cells of the leaf do not dry out uniformly (the marginal cells becoming dry first), the shape of the curves in figure 4 can be explained as the balance between killing or injuring of progressively larger numbers of cells, with initiation of photodestruction in them, and complete drying-out of

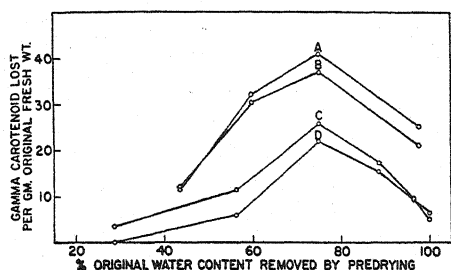


FIG. 4.—Effect of moisture content on photodestruction of carotenoids. Leaves predried *in vacuo* at room temperature before exposure. Carotenoid content of leaves in light subtracted from carotenoids in matched sets in dark to give amount of photodestruction. A and B, carotenol and carotene, respectively, for samples prepared from leaves picked at 2:00 P.M. C and D, carotene and carotenol, respectively, for samples prepared from leaves picked at 9:00 A.M. Light intensity, 1000 foot-candles; exposure period, 3 hours.

progressively larger numbers of cells, with concomitant decreases in rate of photodestruction in such cells.

TEMPERATURE AND CAROTENE DESTRUCTION.—Temperature characteristics for the enzymic oxidation of carotenoids are given in figure 5. Matched sets of fresh leaves and autoclaved leaves (as controls) were prefrozen, brought to the desired temperatures quickly by dipping in water slightly warmer than these temperatures, and incubated for 1 hour at the different temperatures. The maximum in the curve is a result of two opposing effects—the increase in rate of carotene destruction with higher temperature and the increase in rate of en-

zyme inactivation with rising temperature. For the time interval employed, the maximum enzymic destruction of carotene was obtained at approximately 37° C. The temperature coefficients for a 10° rise in temperature (Q_{10}), calculated for the ranges of 4°–15° C. and 15°–24° C., are 1.68 and 1.59, respectively. These values are reasonable for enzymic reactions (15). It should be noted that at 80° C. there was appreciable carotene destruction which indicates that the destruction of the enzyme even at this temperature is by no means instantaneous.

The effect of temperature on the rate of photodestruction of carotenoids was determined. Since in autoclaved leaves a wide temperature range can be used without fear of disrupting the system, rates of photodestruction were determined at only two widely separated

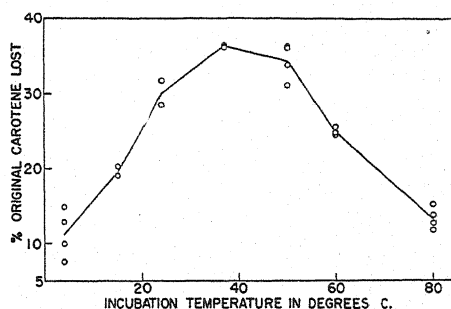


FIG. 5.—Effect of temperature on enzymic destruction of carotene in prefrozen leaves. Carotene loss calculated on basis of carotene content of blanched controls. Incubation time, 1 hour.

temperatures. Matched sets of leaves were exposed at 24° C. and 64° C. in light and in dark by floating the leaves on water maintained at these temperatures. Light intensities (mercury vapor lamp) of 200 foot-candles (light limiting) and 1400 foot-candles were used. In replicated experiments the Q_{10} for both light intensities calculated from observations at these two temperatures was 1.2–1.3. This

temperature coefficient falls within the range of coefficients for photochemical reactions in which temperature affects some nonphotochemical step in the reaction (3). Since the temperature coefficient of photodestruction is low, adequate control of temperature was easily obtained by performing experiments in a room in which fluctuations were limited to 1° or 2° C.

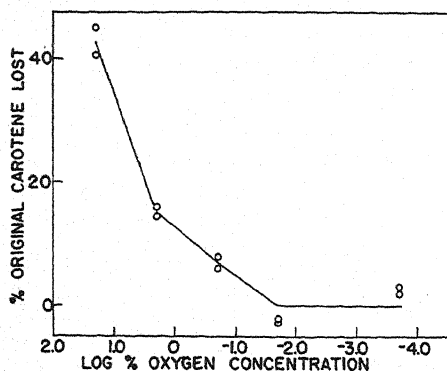


FIG. 6.—Effect of oxygen concentration on enzymic destruction of carotene in prefrozen leaves. Incubation time, 2½ hours; temperature, 37° C.

OXYGEN AND CAROTENE DESTRUCTION.—The relation of molecular oxygen to enzymic destruction of carotene was investigated. Matched leaf samples were placed in 8-oz. glass bottles. The oxygen concentration was adjusted by evacuating to a known pressure and restoring to atmospheric pressure with tank nitrogen scrubbed free of oxygen with alkaline pyrogallol. By repeating this process, the oxygen tension could be reduced to any desired value. Fresh leaf sets were frozen in the bottles after the desired oxygen concentration had been obtained. Control samples (autoclaved leaf sets) were treated exactly the same as the fresh leaves. After freezing, leaves were incubated at 37° C. for 2½ hours. Figure 6 shows that enzymic destruction of carotene is reduced at lower oxygen tensions

and that it falls to zero in the region of 0.02–0.2% oxygen. This proves that enzymic destruction of carotene is oxidative and that molecular oxygen is necessary for the process. This is in interesting contradistinction to the photodestruction of carotene in which some destruction occurs in the absence of molecular oxygen (fig. 7). It would appear that the decolorization of carotene by substances other than oxygen is activated by light.

In studying oxygen tensions and photodestruction, leaf sets were placed in the bottles so that they could be subsequently exposed to light. They were then autoclaved, and the oxygen tension lowered to the desired values. The samples were exposed to 900 foot-candles for 2–4 hours, the time varying in different ex-

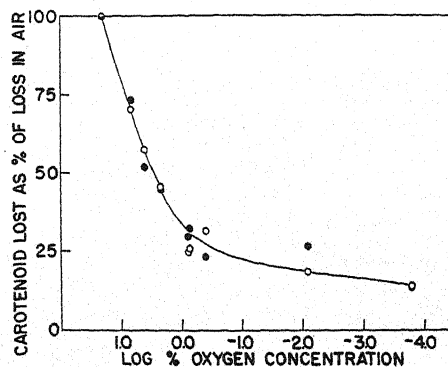


FIG. 7.—Effect of oxygen concentration on photodestruction of carotenoids in autoclaved leaves. Open circles, carotene; closed circles, carotenol. Light intensity, 900 foot-candles; temperature 23° C; exposure period, 3 hours.

periments. In a series of experiments (table 2) leaves in an atmosphere of nitrogen lost $23.9 \pm 3.5\%$ as much carotene and $18.9 \pm 2.1\%$ as much carotenol as the samples in air (means of five determinations). Figure 7 gives the results of a series of experiments in which intermediate oxygen tensions were employed. Between 20% and 0.5–1.0% oxygen, the photodestruction was proportional to the

logarithm of the oxygen concentration (11, p. 530 [on relation of oxygen tension to photo-oxidation and respiration]). Below 0.5% oxygen, photodestruction decreased only slightly with decreasing oxygen tensions. In several experiments (II and III, table 2) it was found that in nitrogen as much photodestruction occurred between 2 and 4 hours as occurred in the first 2 hours. If photodestruction of carotenoids in the absence of molecu-

Leaves were floated on water and exposed for 6 hours to different intensities of light by varying the distance from the light source (mercury vapor lamp). Control sets were analyzed after autoclaving. The results (fig. 8) indicate that up to about 300 foot-candles (measured by Weston meter), photodestruction is roughly proportional to light intensity. Increasing the light intensity above 300 foot-candles results in increased photo-

TABLE 2
PHOTODESTRUCTION OF CAROTENOID PIGMENTS IN AUTOCLAVED LEAVES IN AIR
AND IN N₂. CAROTENOIDS DESTROYED IN LIGHT EXPRESSED AS PERCENTAGE
OF CAROTENOID CONTENT OF DARK CONTROLS

EXPERIMENT	ATMOSPHERE	LIGHT INTENSITY (FOOT-CANDLES)	DURATION (HOURS)	% PIGMENT LOST		LOSS IN N ₂ AS PERCENTAGE OF LOSS IN AIR	
				Carotene	Carotenol	Carotene	Carotenol
I.....	Air	1400	3	76.5	53.0
	N ₂	1400	3	19.0	10.3	24.9	19.4
II.....	Air	1000	2	48.0	31.0
	N ₂	1000	2	11.5	6.4	24.0	20.6
	Air	1000	4	80.0	62.4
	N ₂	1000	4	27.8	16.0	34.8	25.7
III.....	Air	1000	2	44.1	28.8
	N ₂	1000	2	5.6	4.2	12.7	14.6
	Air	1000	4	58.9	38.4
	N ₂	1000	4	13.6	5.4	23.1	14.1
Means ± S.E.....						23.9 ± 3.5	18.9 ± 2.1

lar oxygen is oxidative, some cellular constituents may function as oxidants in place of oxygen; it is also possible that photodestruction of carotenoids in absence of oxygen may proceed through reduction of carotenoids rather than oxidation (11, p. 505). However, under such conditions, the rate of photodestruction is only about 20% of what it is in air.

LIGHT INTENSITY, LIGHT QUALITY, AND PHOTODESTRUCTION.—The relationship of light intensity to photodestruction was investigated with autoclaved leaves early in the course of these studies.

destruction but not in the same proportion observed below 300 foot-candles. In most of the work on photodestruction, light intensities of 1000–1500 foot-candles have been used, since an appreciable amount of photodestruction will occur in a few hours at these intensities.

The effect of light quality on photodestruction was determined by interposing dye solutions between the light source (a 1000-watt tungsten-filament lamp) and the autoclaved leaf samples. A primary filter of copper acetate solu-

tion was used to filter out the infrared radiation and reduce the proportion of energy in the red end of the spectrum. Transmittancies of the dye solutions and copper acetate were determined with a Beckman spectrophotometer (model 11, 1-cm. cells) and are given in figure 10. The yellow-green filter transmitted some light in the blue-violet region, but this contamination is unimportant, since the

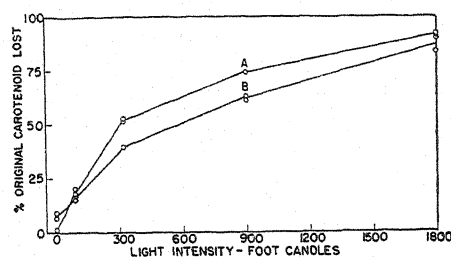


FIG. 8.—Light intensity and photodestruction in autoclaved leaves. Matched sets analyzed for original carotenoid content, after autoclaving. A, carotene; B, carotenol. Duration of experiment, 6 hours; temperature, 23°–25° C.

output of the light source used in the experiment was so low in the blue-violet region. By varying the concentration of the dyes, the light energy transmitted by each filter was equalized. Light energy was measured with a calibrated thermopile and was 100 or 150 μ watts/sq. cm., varying in different experiments. By spectroscopic observation (Hilger spectrometer) of transmitted light from the light source, the bands reported in table 3 were visible.

The results (table 3) show that photodestruction occurred in the orange and yellow-green regions of the spectrum as well as in the blue. Since carotenoid pigments do not absorb light in the yellow-green or orange regions, it seemed likely that the light was being absorbed by chlorophyll, which then directly or indirectly caused the oxidation of the carotenoids.¹ This view was supported by the

fact that photodestruction by yellow-green light was somewhat less than that caused by blue or orange light. An experiment (III, table 3) was then run with autoclaved, etiolated bean leaves, which, by measurement of light absorption of extracts at 670 m μ , were shown to contain only about 1–2% as much chlorophyll as normal bean leaves. In this case, owing to the low initial carotene content of the leaves, the carotenol data only are reported. Despite the low chlorophyll content of the leaves, photodestruction of carotenol occurred in all three regions of the spectrum. The original carotenol content was approximately 50% as high as in green leaves. Photodestruction of carotenols (experiment II, table 3) amounted to about 10%. Allowing for the longer time interval used in experi-

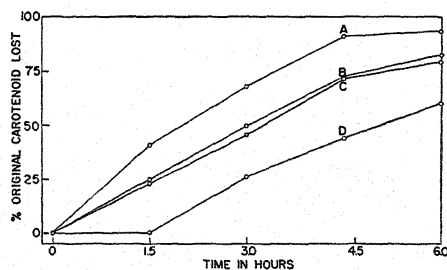


FIG. 9.—Effect of leaching autoclaved leaves on subsequent rates of photodestruction of carotenoids. Difference between carotenoid content of dark controls and light-treated sets expressed as percentage of carotenoids in controls. A, carotene loss in leached leaves; B, carotene loss in unleached leaves; C, carotenol loss in leached leaves; D, carotenol loss in unleached leaves. Light intensity, 1200 foot-candles; temperature, 23°–24° C.

ment III, the amount of carotenol destroyed, on an absolute basis, compares fairly well with the amount of photodestruction in experiment II. It would seem, therefore, that if chlorophyll is the light-absorbing agency in the photodestruction of carotenoids, much lower

¹ Cf. FRANCK and FRENCH (2) on photautoxidation in blue and red light.

concentrations of chlorophyll than normally occur in green leaves are effective in promoting photodestruction.

EFFECT OF LEACHING WITH WATER ON PHOTODESTRUCTION.—From early experiments on the effect of the time factor on photodestruction, it was suspected that natural antioxidants in the leaf were retarding photodestruction of the pigments. An experiment was run in which

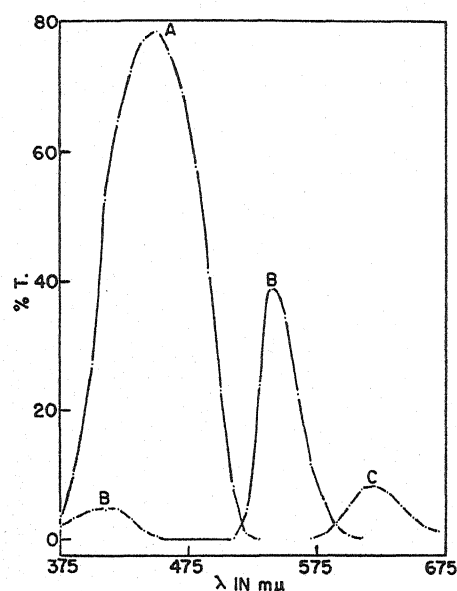


FIG. 10.—Transmittance of dyes used in study on effect of light quality on photodestruction, determined by Beckman spectrophotometer. Results calculated for combined absorption by primary filter (1 cm. 2% copper acetate) and 1 cm. of following dye combinations: A, 0.01% Pontamine Fast Turquoise 8GL and 0.005% Victoria Pure Blue BO; B, 0.01% Pontamine Fast Turquoise and 0.01% Orange G (Du Pont); C, 0.02% Orange G and 0.02% Fast Red (Coleman-Bell).

the photodestruction in leaves blanched by boiling water for 5 minutes (leached material) was compared with that in leaves blanched by steam (unleached material). The photodestruction of carotene after $1\frac{1}{2}$ hours was almost twice as high in the leached material as in the unleached. A time study was made in which

half the samples were blanched dry in the autoclave and the other half blanched in preheated water in the autoclave. The latter were then soaked for several hours in cold water to assure complete leaching of water-soluble constituents. The leaf sets, covered with mineral oil, were exposed to light, with controls kept in the dark. The results show (fig. 9) that, during the first $1\frac{1}{2}$ hours, the carotene loss in leached leaves was 60% higher than in unleached leaves. Carotenols were not photodestroyed at all in unleached leaves during this period, while appreciable photodestruction occurred in leached leaves. After the first $1\frac{1}{2}$ hours, the rates of photodestruction were the

TABLE 3
PHOTODESTRUCTION OF CAROTENOID PIGMENTS BY LIGHT OF DIFFERENT SPECTRAL REGIONS

SPECTRAL REGION IN Mμ	% CAROTENE DESTROYED IN GREEN LEAVES		% CAROTENOL DE- STROYED IN ETIOLATED LEAVES
	Experi- ment I*	Experi- ment II*	Experi- ment III*
430-505.....	16.9	16.7	43.6
520-590.....	15.1	14.1	33.2
600-640.....	17.5	20.0	45.4

* Conditions of experiments: I, duration, 6 hours; light energy, 150 μ watts/sq. cm.; II, duration, 9 hours; light energy, 100 μ watts/sq. cm.; III, duration, 18 hours; light energy, 100 μ watts/sq. cm.

same in leached and in unleached leaves. A definite induction period owing to the antioxidant action of water-soluble constituents of the leaf has been demonstrated.²

Discussion

The marked effect of the water content of partially dried leaves on the en-

² Cf. RABINOWITCH (11, p. 526) on photo-oxidation of cellular oxidation substrates in preference to oxidation of pigments.

zymic destruction of carotene has been demonstrated (fig. 3). Bean leaves which were 85% dehydrated lost carotene at almost twice the rate of loss observed in leaves which were 75% dehydrated. As complete dryness is approached, the rate of change in enzyme activity is even more pronounced, with completely dry leaves showing no enzymic destruction of carotene during incubation. We have referred previously to the experiment of MITCHELL and HAUGE (10), in which carotene loss in leaves drying in the dark is used as a measure of enzymic destruction in leaves drying in the light. From the above considerations, it can be seen that small differences in rates of drying may result in different amounts of enzymic destruction in the light and in the dark, giving erroneous results in the calculated amount of photodestruction.

Furthermore, small fluctuations in the rate of drying of leaves exposed to similar conditions of light and temperature may result in considerable variations in the relative importance of enzymic destruction and photodestruction because of the differential effect of moisture content on these two processes (figs. 3, 4). For example, leaves which have lost 75% of their water will have a maximum rate of photodestruction of carotenoids, while leaves which have lost 85% of their water will exhibit a maximum rate of enzymic destruction. The relative periods of time that leaves remain at these moisture contents will influence the relative importance of these two processes.

The influence of light on leaf temperature may further invalidate such studies. In the experiment reported in table 1, the observed temperature of leaves in light was approximately 10° C. above air temperature and leaf temperature in the dark. The higher leaf temperature in the light would influence enzymic destruc-

tion through the effect of temperature per se on enzymic activity and the effect of temperature on the rate of drying. In the present experiments with photodestruction we were able to limit differences in leaf temperature in light and in dark to 1° C., even with light intensities approaching 2000 foot-candles, by using a water filter to reduce infrared radiation and to cool the leaves by reradiation to the cold water.

In our work with bean leaves the duration of exposure of leaves to conditions favoring photodestruction and/or enzymic destruction is a critical factor. The rate of enzymic destruction is not constant, being initially high, and decreasing to virtually zero after some 40-50% of the carotene has been destroyed (fig. 1). Although the photodestructive rate is more regular in autoclaved leaves (fig. 9), it also decreases markedly with time (fig. 2) in unautoclaved (predried) leaves. Photodestruction, however, continues at an appreciable rate after enzymic destruction virtually ceases. The time interval used in comparing relative rates of photodestruction and enzymic destruction will therefore greatly influence the results.

From the data in figure 8, the dependence of rate of photodestruction of carotenoids on light intensity is obvious. Any estimation of the importance of photodestruction would be influenced by the light intensity incident on the exposed leaves. In terms of field practice, the greater retention of carotene in hay dried in the windrow than in the swath (17) is an effect attributable, in part at least, to the lower light intensity incident on much of the hay in the windrow.

Our study of the factors influencing photodestruction and enzymic destruction of carotene has made it possible to measure these processes accurately in

material which, although under controlled environmental conditions, is directly comparable with material under field conditions. By predrying leaves in a vacuum to a given moisture content and maintaining them at such moisture content during the period of a test, we are able to arrest the complex, ever changing conditions which occur in the field and so to study the effectiveness of various environmental and physiological factors on the rate of destruction of the carotenoid pigments. We have further shown that prefreezing is a useful technique for such studies, since material which is more uniform in regard to the condition of the leaf cells is obtained, while the processes of carotene destruction are qualitatively unaltered by the freezing treatment.

The probability that the carotene-destroying processes are indirect improves the likelihood of success in controlling them and in improving the retention of the carotene in field-cured hay crops. MITCHELL and HAUGE (9) have presented evidence that the enzyme involved in the destruction of carotene in leaves may be a lipoxidase. The coupled oxidation of carotene (14, 16) by the lipoxidase-fat system offers greater opportunities for control than if carotene were the direct substrate of enzymic action. Similarly, the fact that light does not act directly on carotene to cause photodestruction improves the possibility of protecting the carotene.

The presence of naturally occurring water-soluble substances which function as antioxidants for the photodestruction of carotene in bean leaves is of interest in several connections. First, the determination of the character of these substances would be of value per se. Second, the observation that bean leaves may differ in their susceptibility to photode-

struction, depending on the time of day the leaves are picked from the plants (fig. 4), may be due to differences in the natural antioxidant content associated with time of day. Hence, in any study on the effect of physiological and environmental conditions on photodestruction and enzymic destruction of carotenoids, due consideration must be given to possible variations in the quantity of natural antioxidants.

Summary

1. The enzymic and photodestructive processes affecting the carotene content of bean leaves have been studied. By pre-treating leaves (drying or freezing) and controlling the conditions of experimental exposure, we have been able to measure quantitatively the separate processes of carotene destruction in leaves in which both enzymic destruction and photodestruction have occurred simultaneously.

2. In partially dried leaves, carotene is destroyed rapidly both by enzyme- and by light-mediated processes until about 50% of the carotene is gone. The rates of destruction, especially the enzymic rate, then decrease, although in the light a further gradual decrease in carotene content has been observed. In autoclaved leaves, however, photodestruction continues at a high rate until 80-90% of the carotene is destroyed. In prefrozen leaves, incubated at 37° C. in the dark, 45% of the carotene is destroyed by enzymic action in 2 hours, but the remaining carotene is stable. Our results indicate that both the photodestructive and the enzymic processes are important in accounting for the losses of carotene which occur in field-curing of hay.

3. As a leaf dries out, the cells in the margin of the leaf suffer injury or death first, and carotene destruction is initi-

ated in progressively increasing numbers of cells. In tests of the enzymic destruction of carotenoids in leaves held at given moisture contents, maximum destruction of the pigments was found in leaves which had lost 80-95% of their water by predrying. In completely dehydrated leaves, no enzymic destruction of carotenoid pigments occurred. Photodestruction also becomes progressively greater in leaves which have decreasing moisture contents and reaches a maximum when about 68-80% of the water had been lost by predrying. Completely dehydrated leaves show a low but distinct rate of photodestruction.

4. Enzymic destruction, in the time interval used, is at a maximum at approximately 37° C. and decreases at higher temperatures. The temperature coefficient (Q_{10}) for enzymic destruction is 1.6-1.7 between 4° and 25° C., and is 1.2-1.3 for the photodestructive process between 24° and 64° C.

5. Atmospheric oxygen is essential for enzymic destruction of carotenoids. In an atmosphere containing 0.02% oxygen or less, enzymic destruction does not occur. Photodestruction is also influenced by atmospheric oxygen, and the rate of photodestruction is proportional to the logarithm of oxygen concentration from about 0.5 to 20% oxygen. Below 0.5% oxygen, further decreases in oxygen tension do not appear to influence photodestruction appreciably. It is suggested

that some cellular constituents can replace atmospheric oxygen in the photodestruction of the carotenoid pigments or that the carotenoids are destroyed by photoreduction in the absence of oxygen.

6. Photodestruction of carotenoids is roughly proportional to light intensity up to approximately 300 foot-candles. Although photodestruction continues to increase at higher light intensities, the relationship is not the same as at low light intensities. Photodestruction has been shown to occur equally well in orange, yellow-green, and blue light, proving that the light energy is not absorbed directly and exclusively by the carotene. The influence of chlorophyll content has been investigated. The photodestruction of carotenols was found to be as great in etiolated leaves (having only 1-2% of the chlorophyll content of normal green leaves) as in green leaves. Furthermore, photodestruction occurred in orange light as well as in blue light in the etiolated leaves.

7. Naturally occurring antioxidant(s) have been demonstrated in bean leaves. An induction period is noted in the photodestruction of carotenols.

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SELECTION AND BREEDING FOR HIGH β -CAROTENE CONTENT (PROVITAMIN A) IN TOMATO¹

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Introduction

In the summer of 1942 a survey for β -carotene content (provitamin A) was made of 240 varieties, wild types, and hybrids of four species of tomato (*Lycopersicon*). The results, showing very high β -carotene content in certain hybrids, were reported in 1943 (1). Subsequent work has been directed toward genetic stabilization of hybrid material with high β -carotene content and the development of corresponding commer-

cial-type varieties. The results of these efforts are reported in this paper.

Methods

Fruits were grown, sampled, and analyzed as described in detail in a previous publication (2). In brief, representative fruits were homogenized in a Waring blender, and a weighed aliquot was extracted with acetone and hexane (75:60). Acetone was removed by washing with water. Xanthophylls and esters were removed with 90% methanol and 20% KOH in methanol. Spectroscopic readings (for β -carotene and lycopene determinations) were made directly on the resultant hexane solution. Check values for β -carotene content were obtained by chromatographic separation and spectroscopic analysis.

¹ Journal paper no. 286 of the Purdue University Agricultural Experiment Station. This investigation was supported in part by a grant from the Nutrition Foundation, Inc., and is a joint contribution of the departments of botany and plant pathology and of agricultural chemistry.

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Results

DISCOVERY AND SELECTION OF HIGH β -CAROTENE LINES

The survey in 1942 of red- and yellow-fruited species of *Lycopersicon* did not reveal any selections remarkably high in β -carotene content. In this survey the 156 red- or yellow-fruited selections, representing the species *L. esculentum* and *L. pimpinellifolium*, possessed a β -carotene concentration ranging from 1.0 to 19.2 γ /gm. and a lycopene content ranging from 0.8 to 463.0 γ /gm. on the fresh-weight basis.⁵ The 23 commercial varieties included in the above selections ranged near the mean of all observations. As determined at that time and in later studies, the green-fruited species *L. peruvianum* and *L. hirsutum* were very low in carotenoids, ranging from 0.7 to 3.6 γ /gm. of β -carotene and from zero to 1.4 γ /gm. of lycopene.

Although the survey of the species and varieties revealed a few selections of promise as parental material in breeding for high β -carotene content, a much better selection was obtained from the back-cross [Baltimore \times F_1 (Rutgers \times *L. hirsutum* P.I. 126445)]. The latter contained three and a half times as much β -carotene (67.5 γ /gm.) as the highest of the red- or yellow-fruited selections. Also, it contained nine times as much β -carotene as the highest, and thirteen times as much as the average, of the commercial varieties.

Selection 4079-5012 is a single-plant selection from the F_3 population [Indiana Baltimore \times F_1 (Rutgers \times *L. hirsutum* 126445)] that has been used extensively as a high β -carotene parent. The original selection is small-fruited and of poor quality and low yield. The fruit size and carotene content of this selec-

tion are given in table 1. The total carotene fraction includes lycopene. Included in the same table are the results of further selection in later generations for high β -carotene content. Of eighteen progeny plants analyzed in the F_4 generation in 1943, plant no. 9 contained the highest content of β -carotene. F_5 and F_6 progenies derived from this plant also contained high concentrations of β -carotene. The selection within line 4079-5012 indicated that the factors primarily responsible for high β -carotene content were fixed relatively early in the selection process, but apparently factors with minor influences on the β -carotene content may still be heterozygous.

TRANSFER OF HIGH β -CAROTENE TO COMMERCIAL TYPES IN THE BREEDING PROGRAM

When the selection 4079-5012 was used in crosses with Baltimore, Indiana Canners strain (cross 4360), fruits of the F_1 plants were intermediate or low in percentage and content of β -carotene (table 2). In the F_2 , however, high β -carotene selections were recovered. The frequency with which high β -carotene lines were obtained from crosses suggests that the number of major factors necessary to obtain high β -carotene content, in addition to those present in Indiana Baltimore, is small. In the F_3 generation of cross 4360, in which selections were made only for the largest-fruited types, the recovery of high β -carotene lines was not so great as expected from the prior work on selecting within line 4079-5012. However, β -carotene has been increased to 83 γ /gm. in selections with fruit size equal to that of many commercial varieties but somewhat smaller than that of the recurrent commercial parents used in this program.

⁵ Gamma (γ) is equivalent to microgram.

A second selection, 4079-5016, has proved better than 4079-5012 in transmitting fruit size to its progenies. This selection has been used extensively in crosses with Rutgers variety. Data are presented in table 3 on the fruit size and β -carotene content of selection 4079-5016, of Rutgers, and of selections within the F_1 and F_2 generation of crosses between Rutgers and 4079-5016. It is apparent that fruit size in cross A4408 is equal to Rutgers in many selections and that β -carotene content averages about twelvefold that of the Rutgers variety.

TABLE 1
 β -CAROTENE CONTENT OF FRUITS IN ADVANCED GENERATIONS
OF SELECTION 4079-5012

YEAR	CROSS	GENERATION	FRUIT WEIGHT (GM.)	CAROTENE FRACTION		
				Total (γ /gm.)	β -carotene	
					%	γ /gm.
1942	4079-5012*†	F_3	16	66	73	49
1943	4079-5012-1*†	F_4	22	48	83	40
	3		24	71	13	10
	5		30	94	14	13
	7		24	64	15	10
	9*		15	85	93	79
	11		51	59	62	37
	13		33	82	34	27
	15		84	82	21	17
	17		24	56	92	51
	Mean of 18		28	63	55	35
1944	4079-5012-9-1†	F_5	21	61	92	56
	3		22	110	97	107
	5		27	76	98	74
	7		15	83	94	78
	9		21	115	96	107
	11		28	71	96	69
	13		35	120	96	115
	15		20	90	97	87
	17		20	101	97	98
	19		22	87	96	83
	21		13	58	93	54
	23		24	100	96	96
	25		24	81	97	79
	Mean of 25		23	88	96	84
1945	4079-5012-9-9-X†	F_6	23	101	88	89
	Mean of 12					
	4079-5012-9-11-X†	F_6	29	83	94	77
	Mean of 10					
	4079-5012-9-13-X†	F_6	34	92	82	79
	Mean of 12					

* Indicates average value of more than one analysis.

† Parentage: [Baltimore \times F_1 (Rutgers \times *L. hirsutum* P.I. 126445)].

TABLE 2
 β -CAROTENE CONTENT OF FRUITS OF BALTIMORE AND OF F₁, F₂, AND F₃
 GENERATIONS OF CROSS OF BALTIMORE WITH HIGH
 β -CAROTENE SELECTION

YEAR	CROSS	GENERA- TION	FRUIT WEIGHT (gm.)	CAROTENE FRACTION		
				Total (γ /gm.)	β -carotene	
					%	γ /gm.
1942	Baltimore (mean of 5)	P	179	63	9	6
1943	Baltimore (mean of 17)	P	136	64	9	6
1945	Baltimore (mean of 9)	P	215	80	4	3
1943	4360-2*	F ₁	130	28	70	20
	4		55	67	9	6
	6		35	66	35	23
	8		188	101	6	7
	10		54	34	58	19
	12		52	45	50	22
	14		44	65	11	8
	16		21	86	59	50
	Mean of 16		68	63	40	22
1944	4360-16-3*	F ₂	70	95	96	91
	6		92	81	91	73
	9		70	101	92	92
	12		74	56	46	26
	15		35	56	71	39
	18		88	70	44	31
	23		86	90	40	36
	27		84	88	32	28
	30		48	55	40	22
	33		47	78	53	41
	36		137	54	28	15
	39		37	43	71	30
	42		39	51	49	45
	45		39	53	44	23
	46		142	58	87	50
	48		26	77	36	28
	51		51	61	25	16
	54		44	113	5	6
	57		72	72	44	32
	62		69	87	36	31
	65		44	98	84	82
	Mean of 65		62	75	52	39
1945	4360-16-3-1*	F ₃	131	90	45	46
	2		159	88	46	41
	3		57	109	94	103
	4		116	99	10	10
	4360-16-6-1*	F ₃	90	112	21	14
	2		194	39	24	17
	3		179	52	90	46
	4		125	113	45	49
	5		64	74	83	61
	6		119	104	80	83
	7		141	88	18	16
	8		129	75	37	28
	4360-16-45-1*	F ₃	107	70	23	9
	2		126	69	54	37
	3		185	74	41	22
	4		95	116	21	25
	4360-16-62-1*	F ₃	125	81	28	23
	3		77	85	20	17
	5		111	104	9	9
	7		122	87	30	26
	9		70	118	14	16
	Mean of 10		115	82	22	17

* Parentage of cross 4360: Baltimore \times F₃ selection 4079-5012. 4079-5012 = [Baltimore \times F₁ (Rutgers \times *L. hirsutum* P.I. 126445)].

INTERRELATIONSHIP OF β -CAROTENE,
LYCOPENE, AND COLOR

In the selections of high β -carotene content the mean concentrations of the total carotenes, calculated as the sum of the concentration of lycopene and β -

carotene, have not been increased over that of Rutgers and Baltimore (tables 2, 3). It appears that the increased concentration of β -carotene has occurred at the expense of lycopene—the carotene primarily responsible for the red fruit color

TABLE 3

β -CAROTENE CONTENT OF FRUITS OF RUTGERS, OF SELECTION 4079-5016, AND OF F_1 AND F_2 GENERATION OF CROSSES BETWEEN THESE TWO PARENTS

YEAR	CROSS	GENERA- TION	FRUIT WEIGHT (gm.)	CAROTENE FRACTION		
				Total (γ /gm.)	β -carotene	
					%	γ /gm.
1942	Rutgers	P	255	56	7	4
1943	Rutgers (mean of 5)	P	125	60	5	3
1944	Rutgers (mean of 3)	P	235	77	3	2
1945	Rutgers	P	252	98	< 1	< 1
1942	4079-5016*	F_1	66	45	54	26
1943	4079-5016-2*	F_1	122	27	93	25
1944	4408-1*†	F_1	160	45	11	5
	2		77	78	47	37
	3		122	94	9	8
	4		171	53	41	22
	5		91	77	37	29
	6		154	53	12	6
	7		138	91	8	7
	A4408-1†	F_1	123	73	46	34
	2		88	85	9	8
	3		138	103	3	3
	4		151	86	12	11
	5		111	114	2	2
1945	4408-2 \times † (mean of 29)	F_2	111	83	37	23
	4408-4 \times † (mean of 8)	F_2	213	77	33	26
	4408-5 \times † (mean of 46)	F_2	147	86	27	22
	A4408-1- 2†	F_2	132	53	72	38
	4		177	81	81	66
	6		176	75	54	40
	8		206	83	61	56
	10		214	104	< 1	< 1
	12		148	79	4	3
	14		141	107	8	8
	16		187	70	32	20
	18		167	72	45	32
	20		242	96	36	35
	22		202	77	87	66
	24		165	69	46	32
	26		134	82	38	31
	28		133	118	2	2
	30		264	147	10	15
	32		220	88	44	40
	34		134	92	18	17
	36		165	63	37	42
	38		121	82	5	4
	Mean of 38		180	81	39	39

* Parentage: [Baltimore \times F_1 (Rutgers \times *L. hirsutum* P.I. 126445)].

† Parentage: Rutgers \times F_4 selection of 4079-5016-2.

in most varieties of tomatoes. Consequently, fruits containing a high concentration of β -carotene are of deep orange color. However, analyses of orange and orange-yellow varieties currently on the market, such as Jubilee and Tangerine, have shown them to be no higher in β -carotene content than the standard red-fruited varieties. Their orange color is due largely to isomers of lycopene which do not have vitamin A activity.

β -carotene per gram of fresh fruit have been obtained.

RELATIVE VALUE OF TOMATOES AS A
SOURCE OF PROVITAMIN A IN
HUMAN DIET

The β -carotene content of several vegetables commonly recommended as excellent sources of provitamin A in the human diet is given in table 4. As a source of provitamin A, tomatoes selected for high β -carotene content are

TABLE 4
 β -CAROTENE CONTENT OF SOME VEGETABLES RECOMMENDED
AS EXCELLENT SOURCES OF PROVITAMIN A

VEGETABLE	β -CAROTENE (γ /GM.)	
	Fresh	Dry
Carrot*	90	900†
Spinach*	54
Sweet potato*	44	120†
Squash, winter*	7
Tomato, commercial*	7	120‡
Tomato, high β -carotene selection.	83	1200

* Values calculated from data presented in *Tables of Food Composition*, published by Committee on Food Composition, National Research Council.

† Values on dehydrated product.

‡ Assume approximately 6% dry matter.

All fruits analyzed in the breeding project have been classified for color. Generally, when the β -carotene content is greater than 35% of the total carotene pigments present, the fruit is classified as orange. Fruits of characteristic tomato redness are relatively low in β -carotene content, at least 65% of the carotenes present being lycopene. Because of present consumer demands for red tomatoes, work is being directed toward developing commercial varieties not only of the highest possible β -carotene content (orange-colored) but also of the highest possible β -carotene content compatible with red fruit color. At the present time, red selections containing approximately 30 γ of

about equal to carrots and will surpass most other foods on a fresh-weight basis. When determined on the dry-weight basis, the superiority of these high β -carotene selections as a source of provitamin A in the diet becomes even more marked.

Summary

1. High β -carotene concentration in tomato fruits has been obtained from selections of the cross [Baltimore \times F₁ (Rutgers \times *L. hirsutum* P.I. 126445)]. One selection from this backcross generation produced an average content of 101 γ /gm. of crude carotene, of which 88% occurred as β -carotene.

2. Large-fruited, high β -carotene selections have been obtained, by further backcrossing to commercial varieties, at a frequency great enough to suggest that the number of major factors necessary to obtain high β -carotene, in addition to those present in commercial varieties, is small.

3. β -Carotene appears to be produced at the expense of lycopene, since the total carotenoid concentration in the high β -carotene selection is not increased over that of the red, low β -carotene parent.

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TEMPERATURE COEFFICIENTS OF CELL ENLARGEMENT¹

MARIAN DELLERS CHAO AND W. E. LOOMIS

Introduction

Cell enlargement has been generally regarded as a relatively simple process involving little or no synthesis of protoplasm, with the increase of cell volume resulting from absorption of water through osmotic or imbibitional forces. THUT and LOOMIS (9), however, observed that the growth rates of several plants showed the temperature coefficients of chemical reactions. Even the final growth of leaves of *Ricinus communis* nearly 40 cm. wide showed a temperature coefficient of 2.0 or more, and this in spite of the bright sunlight and lower daytime humidity which accompanied the higher temperatures. Osmosis, and at least certain types of imbibition, acting alone would show the temperature coefficients of physical reactions with Q_{10} values just above 1.0, where

$$Q_{10} = \frac{\text{Rate at } t + 10^{\circ} \text{ C.}}{\text{Rate at } t^{\circ} \text{ C.}}$$

Q_{10} values of 2.0 or more are taken as evidence that chemical reactions of some sort are limiting for the over-all process. The data presented here support the thesis that the enlargement of individual cells, or the enlargement of organs in which cell number is no longer increasing, is limited by chemical processes, either in the protoplasm, in the cellular membrane, or in the cell wall.

Experimentation

DANDELION SCAPES.—Excised sections of young scapes of *Taraxacum officinale*, taken from directly beneath small buds, were grown in nonsterile solutions of sucrose and indoleacetic acid (IAA). The solutions were allowed to reach the experimental temperatures before the sections were inserted. Whole scapes, together with the attached buds, were brought into a cold room for preparation. Sections 1 cm. long from each of ten scapes were cut and immediately placed in 25 ml. of solution in a Petri dish at the experimental temperature. Solutions

¹ Journal paper no. J-1430 of the Iowa Agricultural Experiment Station. Project no. 678.

were changed daily, and bacterial growth was very slight, the solutions remaining clear and no tissue degeneration becoming apparent within 4-day test periods. Temperature coefficients were calculated for cell elongation. Coefficients for intervals other than 10° were obtained with the formula

$$Q_{10} = Q_{nc} = (Q_n)^c,$$

where n is the actual temperature interval studied and c is equal to $10/n$.

TABLE 1
ELONGATION OF EXCISED, 1-CM. SECTIONS
AND OF EPIDERMAL CELLS OF DANDELION
SCAPE DURING 96 HOURS

TEMPERATURE (° C.)	1% SUCROSE + 1.5 MG. IAA/LITER		DISTILLED WATER	
	Increase in section length	Increase in epi- dermal cell length	Increase in section length	Increase in epi- dermal cell length
0.....	1.18X*	1.02X	1.03X	1.01X
5.....	1.22X	1.27X	1.08X	0.99X
10.....	1.31X	1.17X	1.23X	1.11X
15.....	1.90X	1.56X	1.26X	1.24X
20.....	2.10X	1.83X	1.42X	1.37X
25.....	2.20X	1.90X	1.40X	1.22X
30.....	2.25X	1.71X	1.44X	1.11X

* Each figure is average of ten sections or of eighty cells.

Growth studies had shown that such sections of scapes would elongate two to three times in 4 days at room temperature in these solutions, with concentrations of indoleacetic acid ranging from 1 to 10 mg./l. and sucrose present in 1% concentration. As much as 50% elongation occurred, even in distilled water. The elongation of sections and of epidermal cells of sections of scapes after 96 hours in IAA-sucrose solution and in distilled water at various temperatures is shown in table 1. Even cooled sections placed in a pre-chilled solution at 0° C. showed a small but definite elongation.

In IAA-sucrose solution it is doubtful if any cell division occurred at 0° or 5° C. At temperatures from 10° C. through 30° C., however, the average increase in epidermal cell length was less than the increase in section length, and it is probable that some cell division was occurring. Since the ratio of the increase of epidermal cell length to the increase of section length remained nearly constant within this temperature range, however, higher temperatures apparently increased the rate of cell elongation to the same extent that they increased the rate of cell division.

The temperature coefficients of elongation of the sections and of the epidermal cells in IAA-sucrose solution during the 96-hour test period are shown in table 2. From 0° to 20° C., these Q_{10} values ranged from 1.7 to 4.1 for section and from 2.1 to 8.5 for cell elongation. The coefficients

TABLE 2
TEMPERATURE COEFFICIENTS OF ELONGATION
OF EXCISED SECTIONS AND OF EPIDERMAL
CELLS OF DANDELION SCAPE

TEMPERATURE (° C.)	1% SUCROSE + 1.5 MG. IAA/LITER		DISTILLED WATER	
	Q_{10} for sections	Q_{10} for cells	Q_{10} for sections	Q_{10} for cells
0-10.....	1.7	8.5	7.7	11.0
5-15.....	4.1	2.1	3.2	24.0
10-20.....	3.5	4.9	1.8	3.4
15-25.....	1.3	1.6	1.5	0.9
20-30.....	1.1	0.9	1.0	0.3

for cells are based on increase in average cell length as obtained by direct measurements of eighty cells in each lot with an eyepiece micrometer. They are thus minimum values, since any cell division would decrease the average cell length. These high temperature coefficients indicate that chemical reactions of some type

were limiting influences in the elongation of cells of the scape.

In distilled water scape sections showed a smaller but unmistakable elongation (table 1). It is not clear whether cell division occurred during the 4-day growth period in water, although at temperatures of 25° C. and 30° C. the data suggest that it did. In general, the elon-

TABLE 3

ELONGATION OF EXCISED, 1-CM. SECTIONS AND OF EPIDERMAL CELLS OF DANDELION SCAPE IN 1% SUCROSE SOLUTION CONTAINING 5 MG. IAA PER LITER

TEMPERATURE (° C.)	24-HOUR PERIOD		36-HOUR PERIOD	
	Increase in sec- tion length	Increase in cell length	Increase in sec- tion length	Increase in cell length
0.5.....	1.10×	1.38×	1.12×	1.00×
5.0.....	1.13×	1.32×	1.25×	1.02×
10.0.....	1.27×	1.14×	1.26×	1.26×
14.0.....	1.50×	1.66×	1.50×	1.17×
20.5 (21.0)†	1.60×	1.70×	1.60×	1.81×
23.0.....	1.53×	1.81×	1.48×	1.74×
27.0 (28.0)	1.87×	1.92×	1.78×	1.90×
31.0 (32.0)	1.60×	1.99×	1.93×	2.22×
35.0 (34.0)	1.53×	1.33×	1.65×	1.88×

* Each figure is average of ten sections or of eighty cells.

† Values in parentheses are temperatures during 36-hour test where they differed from those of 24-hour test.

gation in distilled water was much less than in the IAA-sucrose solution, and it is more difficult to distinguish the effects of temperature from the natural variation in the scapes. Nevertheless, high temperature coefficients of cell elongation were found from 0° to 20° C. (table 2), even in the absence of an external source of sucrose and auxin.

To reduce the possibly complicating effects of cell division in the excised sections, a second group of experiments was completed in periods of 24 and 36 hours (table 3). During these shorter periods no evidence of cell division was obtained, cell elongation being of the same magni-

tude as section elongation, within the limits of variability of the materials used. In the range from 0° to 20° C., the temperature coefficients (table 4) were again above 2.0 in both experiments, indicating that the limiting process or processes at these temperatures were chemical. The drop in the growth rates at temperatures above 20° C., with less actual growth above 30° to 32° C., is probably related to a low optimum temperature for growth but may be affected also by high respiration and low rates of gas exchange in the partially submerged sections. High temperatures caused injury, as indicated by loss of turgidity and discoloration of scape sections, at temperatures above 32° C.

CASTOR BEAN LEAVES.—It is reasonably certain that cells are no longer dividing in nearly grown leaves of *Ricinus*

TABLE 4

TEMPERATURE COEFFICIENTS OF CELL ELONGATION IN SECTIONS OF DANDELION SCAPE IN SUCROSE-IAA SOLUTION (CALCULATED FROM CHANGES IN SECTION LENGTH)

Temperature range (° C.)	Q ₁₀ (24-hour period)	Q ₁₀ (36-hour period)
0.5-10.0.....	2.84	2.16
5.0-14.0.....	4.45	2.16
10.0-20.5 (10.0-21.0)....	2.13	2.14
14.0-23.0.....	1.07	0.96
20.5-27.0 (21.0-28.0)....	1.77	1.45
23.0-31.0 (23.0-32.0)....	1.17	2.09
27.0-35.0 (28.0-34.0)....	0.54	0.73

communis and that expansion of the blade in such leaves occurs through enlargement of cells only. The temperature coefficients of cell enlargement can thus be obtained from the rates of leaf growth at different temperatures. Young plants, which had been grown in large pots in the greenhouse, were placed in constant-temperature incubators in darkness. The data of table 5 show the enlarge-

ment of nearly grown leaves at three temperatures. Growth rates were satisfactorily high during short periods in the dark but tapered off sharply when the plants remained without light for longer intervals. The Q_{10} values (table 6) in both temperature ranges were high (2.0+) in all three experiments, except in the 22°–

28° C. range of the first experiment, in which moisture may have been limiting (6). In general, these results seem to confirm the findings of THUT and LOOMIS (9) that expansion of such leaves shows high temperature coefficients, suggesting the importance of chemical processes in cell enlargement.

TABLE 5
ENLARGEMENT OF CASTOR BEAN LEAVES AT THREE TEMPERATURES

TEMPERATURE (° C.)	EXPERIMENT I			EXPERIMENT II		EXPERIMENT III (17 HOURS)
	First period (9 hours)	Second period (15 hours)	Third period (11 hours)	First period (14 hours)	Second period (11 hours)	
10.....	0.21*	0.30	0.05	0.22	0.18	0.17
22.....	0.90	0.80	0.33	1.90	0.34	0.65 (20° C.)
28.....	0.96	0.82	0.30	2.72	0.50	2.66 (30° C.)

* Each value is average increase in cm. of lengths of five main veins of a nearly grown leaf on each of three plants.

TABLE 6
TEMPERATURE COEFFICIENTS OF ENLARGEMENT OF CASTOR BEAN LEAVES

TEMPERATURE (° C.)	EXPERIMENT I			EXPERIMENT II		EXPERI- MENT III (17 HOURS)
	First period (9 hours)	Second period (15 hours)	Third period (11 hours)	First period (14 hours)	Second period (11 hours)	
10-22 (10-20).....	3.35	2.26	4.79	5.11	1.64	3.82
22-28 (20-30).....	1.12	1.03	0.85	2.59	2.94	4.09

TABLE 7
ELONGATION OF MARKED SECTIONS OF BEAN HYPOCOTYL AT VARYING TEMPERATURES
DURING 24-HOUR PERIOD IN DARK

SECTIONS (FROM TOP)	TEMPERATURE (° C.)						
	5	10	15	20	25	30	35
1 (0-4 mm.).....	0.5*	0.3	1.5	3.0	1.5	2.3	2.0
2 (4-8 mm.).....	0.0	1.0	1.0	4.5	2.0	2.5	2.5
3 (8-12 mm.).....	0.0	0.0	0.5	6.0	2.0	2.0	2.5
4 (12-16 mm.).....	0.0	0.0	0.5	7.5	1.8	2.3	8.5
5 (16-20 mm.).....	0.0	0.0	1.5	5.8	3.8	4.5	7.8

* Figures are average mm. increases in length of sections originally 4 mm. long. Two seedlings at each temperature except 5° and 15° C. with one seedling each.

BEAN HYPOCOTYLS.—The growth of hypocotyls of *Phaseolus vulgaris* at different temperatures was observed in small seedlings which had been germinated in the dark in sand, marked into uniform regions with India ink, covered with black paper cones to exclude light, and placed at temperatures ranging from 5° C. to 35° C. It was determined that cell division in the hypocotyl was limited to the upper 2–3 mm. (section 1). By calculating the rates of section elongation in regions of the hypocotyl in which cells were no longer dividing (sections 2 through 5), the influence of temperature on cell elongation was measured (table 7). Each section was 4 mm. in length at the beginning of the 24-hour test period. In general, maximum elongation of the sections occurred at 20° C.

In sections 2 through 5, in which growth was assumed, on the evidence from parallel plants, to have wholly resulted from elongation of cells, high temperature coefficients were found for the ranges 10°–20° and 15°–25° C. (table 8). The Q_{10} values for these ranges were all over 2.0. In the range 20°–30° C., the values were less than 1.0, possibly indicating that cell moisture was not maintained in the exposed sections. During a 72-hour growth period, the temperature coefficients of section elongation showed the same trends. Q_{10} values of cell elongation (sections 2–5) were above 2.0 in the temperature ranges 10°–20° C. and 15°–25° C. In the range 20°–30° C., the coefficients on the whole were low (below 1.0).

Some of the irregularity of results may be attributed to the small number of plants used in any one experiment and, for the data shown in tables 7 and 8, to the exceptional growth made by the plants at 20° C. The conclusion from all the experiments, however, is clearly that

there is a high temperature coefficient for cell elongation in bean hypocotyl up to temperature values of 30° C.

Discussion

Chemical reactions affecting the rate of cell enlargement might be localized in the cytoplasm, in the cell membrane, or in the wall. One of us has shown (3) that enlargement in the cells of the cotyledon of *Brassica oleracea* var. *italica* may be

TABLE 8

TEMPERATURE COEFFICIENTS OF ELONGATION OF MARKED SECTIONS OF BEAN HYPOCOTYL DURING A 24-HOUR PERIOD OF GROWTH IN DARK

TEMPERATURE RANGE (° C.)	SECTION				
	1	2	3	4	5
5–15.....	3.00
10–20.....	10.00	4.50
15–25.....	1.00	2.00	4.00	3.60	2.51
20–30.....	0.78	0.56	0.33	0.31	0.78
25–35.....	1.33	1.25	1.25	4.72	2.05

checked by a deficiency of soil nutrients and be resumed if these elements are again made available. At least an indirect effect of a protoplasmic reaction is indicated. In the experiments with *Taraxacum* scapes reported here, however, rapid cell enlargement and some cell division occurred without the further addition of nitrogen or other soil nutrient elements. Studies with attached dandelion scapes (3) showed that early rapid growth was accompanied by a marked increase of protein nitrogen per cell but that the same fraction decreased on a per cell basis during the second spurt of growth at the time of fruit maturity. It thus seems probable that any relationships between protoplasm synthesis and cell enlargement are indirect rather than direct. The relationships shown by

AVERY *et al.* (1, 2) between protein synthesis and hormone synthesis could be a factor in cell enlargement, and it is probable that respiratory energy is either a directly or indirectly contributing factor.

Marked temperature effects on the shrinking and swelling of plant tissue in water have been noted by a number of authors. DELF (4), studying the influence of temperature on the permeability of protoplasm to water, found that the permeability of dandelion scape cells to water, as measured by rate of tissue shrinkage in sucrose solutions, was doubled or tripled with a 10° rise in temperature between 10° and 40° C. STILES and JORGENSEN (8) studied the influence of temperature on the swelling of potato tissue in water and calculated that the Q_{10} values for this reaction ranged from 2.7 to 3.0 between 10° and 30° C. The rate of swelling of carrot tissue in water within the same temperature range showed coefficients of 1.3-1.6. The process of water intake into potato tuber tissue was further investigated by REINDERS (7), who found that absorption of water by aerated slices was stimulated by indoleacetic acid, the reaction having a Q_{10} of 2-3. The effect of temperature on the swelling of mature storage tissue is probably complex, and even DELF's experiments may involve more than simple permeability changes. A possible effect of temperature on permeability would seem, however, to be a factor which should be considered in explaining the effects of temperature on cell enlargement.

Advocates of the theory of growth by intussusception might be of the opinion that a high temperature coefficient is evidence for this mechanism. Any system of adding cellulose or pectic units to an extending cell wall, however, or any growth-hormone action increasing the plasticity of the cell walls (5), would involve enzymatically controlled, chemical reactions. The present evidence does not justify the choice of any one of them.

Whatever reaction or group of reactions may prove to be concerned, the data obtained indicate that chemical reactions are generally limiting for cell-enlargement processes. Such a conclusion does not, of course, eliminate the presence of physical forces, but it does indicate that they are not controlling in the tissues studied.

Summary

The enlargement of nondividing cells of scape sections of *Taraxacum officinale* in a sucrose-hormone solution, of cells of attached, nearly grown leaves of *Ricinus communis*, and of hypocotyls of *Phaseolus vulgaris* seedlings, showed the temperature coefficients of a chemical reaction over temperature ranges between 0° and 30° C. Such Q_{10} values are taken as evidence that chemical reactions in the protoplast and/or the extending wall, rather than the physical processes of osmosis or imbibition, are limiting for cell enlargement under conditions of an adequate moisture supply.

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RELATION OF LIGHT INTENSITY TO EFFECT OF 2,4-DICHLORO-PHENOXYACETIC ACID ON WATER HYACINTH AND KIDNEY BEAN PLANTS

WILLIAM T. PENFOUND AND VIRGINIA MINYARD

In one study on the effect of formagenic herbicides on water hyacinth, we utilized the droplet method. The use of droplets of the butyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) in kerosene proved very advantageous because of the rapid spreading of the droplets, the speedy entrance of the material into the tissues, and the relatively rapid destruction of the organism. Tests with droplets of kerosene alone showed only a very slight necrosis at the site of application.

In one experiment much greater epinasty of leaves and subsequent necrosis developed in the laboratory than in full sunlight. Thinking that light intensity might be a determining factor, we treated sets of plants in the laboratory, under a table out of doors, and on top of a table out of doors. In a somewhat different experiment leaves of parent plants having three offshoots were treated in the laboratory and under field conditions. In both experiments the plants were similar in size and were removed from the same habitat (tubs in full sunlight) just prior to application of the herbicide and transferred to the experimental condition desired. With all treatments the same pi-

pette was used in order to obtain uniform droplet size and the same concentration (1000 p.p.m.) of the herbicide. Each plant was treated with four droplets, one on each of four different leaves, giving a total of 200 μ gm. per plant.

Daily observations were made on the degree of epinasty and on the percentage destruction. The results on water hyacinth (table 1) include the data at the end of the experiments (14 days) only. Greater epinasty and much greater necrosis occurred in shaded conditions, whether small, medium, or large plants were employed. Complete destruction did not occur in any plants in full sunlight, although several leaves of small plants exhibited considerable necrosis. In the shade the parent plant was killed quickly, and all the offshoots showed considerable necrosis by the end of the experimental period. In full sunlight, however, only a few leaves of the parent plant were killed, the offshoots not only being uninjured but increasing in number by 300%.

It is obvious that these observations concerning the apparent influence of light on the responses of water hyacinth

to the butyl ester of 2,4-D in kerosene differ conspicuously from those of MITCHELL and BROWN (1) and WEAVER and DE ROSE (2). MITCHELL and BROWN found that no bending of the stems occurred when leaves of snap bean plants growing in darkness or in shade were treated with aqueous solutions of 2,4-D in Carbowax 1500. WEAVER and DE ROSE found that "most stem curvature occurred in plants growing in the light" when Red Kidney beans were treated with the ammonium salt of

with four drops of the butyl ester of 2,4-D in kerosene at a strength of 1000 p.p.m. The starch-present and starch-free plants were then divided into three groups of four plants each, these groups being disposed on the same table and exposed to light as follows: (A) by a window with a southwest exposure; (B) in diffuse light; and (C) in darkness. The treatments under each light condition were as follows: (a) control; and two drops each on (b) cotyledons, (c) primary leaves, and (d) primary leaves of plants

TABLE 1
RELATIVE EFFECT OF BUTYL ESTER OF 2,4-D IN KEROSENE (FOUR DROPS OF 1000 P.P.M. PER PLANT) ON SMALL, MEDIUM, AND LARGE PLANTS OF WATER HYACINTH IN DIFFUSE LIGHT AND IN FULL SUNLIGHT (14 DAYS AFTER TREATMENT)

PLANT	EPINASTY		NECROSIS (%)	
	Shade	Sunlight	Shade	Sunlight
Small (leaves 14 in.).....	Marked	Marked	90	30
Medium (leaves 21 in.).....	Marked	Moderate	40	5
Large (leaves 44 in.).....	Marked	Slight	30	None
Medium plants with offshoots.....	Marked	Moderate	100 (parent); 30 (offshoot)	20 (parent); 0 (offshoot)

2,4-D. In all our work, however, the degree of epinasty and the destruction of water hyacinth plants were greater in the shaded plants.

Since it was felt that the greater destruction of water hyacinth in shade (versus sunlight) might indicate a specific difference, we tested Red Kidney beans with the same phytocide. Twenty-four plants in the late cotyledon stage were potted, allowed to develop for 3 days, and then divided into two groups, one of which was placed in full sunlight and the other in darkness for 48 hours. Upon testing, a moderate amount of starch was found in the leaves of those exposed to sunlight, but none was found in plants in darkness. Each plant was then treated, under identical conditions,

from which cotyledons had been removed.

In about 3 hours inrolling was quite evident on all treated leaves. In 6 hours some bending occurred in the first internodes, appearing first on those plants in which the cotyledons were treated. There was also considerable epinasty or hyponasty of the primary leaves, but, since bending was either inward or outward, this phenomenon was not used as a measure of phytocidal activity. Local necrosis of leaves occurred in about 24 hours, but general necrosis did not supervene until the third day. We have utilized the bending of the first internode and the death of plant parts as our major criteria of phytocidal effectiveness.

In all cases the controls showed no ab-

normalities, even in darkness. The reactions of plants on which only the cotyledons were treated were similar to those of leaf-treated plants except that bending occurred slightly earlier and inrolling of the leaves developed slightly later. From our observations it appears that there is no basic difference in phytocidal reaction between cotyledon-treated and leaf-treated plants with the materials and dosages utilized. Removal of the cotyle-

difference among those in direct light, diffuse light, and darkness except that the starch-free plants in direct light fared somewhat better than the others (table 2). With one exception (starch-present plants in darkness) plants in darkness and diffuse light were killed more readily than those in direct sunlight. This checks closely with our experience with water hyacinth under both laboratory and field conditions.

TABLE 2

RELATION OF LIGHT TO PHYTOCIDAL EFFECTIVENESS (ON RED KIDNEY BEAN PLANTS) OF BUTYL ESTER OF 2,4-D IN KEROSENE

DAYS AFTER TREATMENT	DIRECT LIGHT		DIFFUSE LIGHT		DARKNESS	
	Bending	Necrosis (%)	Bending	Necrosis (%)	Bending	Necrosis (%)
In light before treatment (starch in leaves)						
1.....	10°	None	45°	None	30°	None
3.....	90	30	80	30*	45	35
7.....	90	90	*	*	80	80
In darkness 48 hours before treatment (no starch)						
1.....	5°	None	10°	None	30°	None
3.....	20	10	180	70*	90	30*
7.....	30	70	*	*	*	*

* Dead, prostrate.

dons apparently had no effect whatever on the reactions of plants to the phytocide. We are, therefore, presenting only the data on the entire plants in which the primary leaves were treated (table 2).

Bending occurred in nearly all treated plants by the end of the first day (table 2). With the concentration used, it increased with time and was followed by considerable necrosis by the third day. By the end of the seventh day nearly all the plants were dead, and there was little

The reasons for the difference between our results and those of MITCHELL and BROWN (1) and WEAVER and DE ROSE (2) when using the same or very similar species are not entirely clear. We have read their reports carefully and are convinced of the care of their experimentation and of the accuracy of their results. It is possible that the greater amounts of materials used in our experiments (200 μ gm. versus 10-50 μ gm. of herbicide per plant) may account in part for the difference. That the stimulus travels with

soluble carbohydrate is called into question, however, since the greatest bending and the greatest destruction of our Red Kidney bean plants occurred in starch-free plants in diffuse light and in darkness. In one experiment WEAVER and DE ROSE found that one drop of an aqueous solution of the ammonium salt of 2,4-D (1000 p.p.m.) did not induce stem curvature when applied to the cotyledons of the soybean except when they were scratched. This was not true for our Red Kidney bean plants when treated with four drops of the butyl ester (1000 p.p.m.). It is possible that the diversity in results was due to a specific difference, to the greater total amount of 2,4-D used by us, or to the fact that the butyl ester penetrated the cotyledons much more readily than the ammonium salt. In any event, it is certain that the butyl ester of 2,4-D in kerosene is effective on cotyledons and in darkness as well as in sunlight. These facts may be related to the greater penetrating capacity of the carrier (kerosene).

Summary

1. Water hyacinth plants in shade and full sunlight were treated with four drop-

lets each (1000 p.p.m.) of the butyl ester of 2,4-dichlorophenoxyacetic acid (2,4-D) in kerosene.

2. Greater epinasty and much greater necrosis occurred in plants under shaded conditions than in those placed in full sunlight.

3. Red Kidney bean plants were treated with four droplets each (1000 p.p.m.) of the butyl ester of 2,4-D in kerosene, either on the cotyledons or on the primary leaves, and placed in darkness, in diffuse light, or in direct sunlight.

4. Bending and necrosis were similar in cotyledon-treated and leaf-treated plants and in darkness, diffuse light, and direct sunlight except that survival was somewhat better in direct sunlight.

5. It is suggested that the effectiveness of the phytocide used in this experiment may have been enhanced by the penetrating capacity of the carrier (kerosene).

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CURRENT LITERATURE

Growth Regulators for Garden, Field, and Orchard. By JOHN W. MITCHELL and PAUL C. MARTH. Chicago: University of Chicago Press, 1947. Pp. 129+16 figs.+5 tables. \$2.50.

During the past decade progress in the study of the effects of organic chemicals, often referred to as synthetic plant hormones or growth-regulating substances, has been phenomenally rapid. There is scarcely a phase of plant production in which the use of growth-regulating substances has not already become of significance. The field of their use is already a very broad one and is rapidly expanding. The future use and application of growth-regulators may eventually extend into every phase of plant production, storage, and utilization where living plants are concerned.

Up to the present time information concerning them has been available only through technical papers or a few popular articles widely scattered in publications dealing with plant culture. The present book is most timely in that it has been written by two of the most active contributors on both the theoretical and the applied aspects of the use of growth-regulators. It furnishes in the applied field an authentic summary of the information available up to date in clear, direct style and language. Chapters are devoted to weed control, vegetative propagation and transplanting, the preventing of growth in stored plant material, the prevention of premature dropping of fruits, the improvement of fruit-set and the production of seedless fruit, and a composite chapter dealing with other plant responses to these compounds. There is a concise and comprehensive index.—E. J. KRAUS.

Heredity and Variation in Microorganisms. ("Cold Spring Harbor Symposia on Quantitative Biology," Vol. XI.) Cold Spring Harbor, N.Y.: Biological Laboratory, 1946. Pp. 314. \$6.00 (plus postage).

The wide scope of this series of papers and the intensive treatment afforded each of the many aspects of heredity and variation in micro-organisms impose on the reviewer an exceedingly difficult task of summation and abstraction; on the other hand, the inclusion in the volume of the comments and criticisms by those attending the symposium and best qualified to criticize relieves him in large part of his other important function, that of evaluation.

The Cold Spring Harbor Symposium of 1946 comprises, as usual, a comprehensive and up-to-the-minute review of the designated field of the biological

sciences by its outstanding experimentalists. The individual papers are short and concise, and for the most part the material is exceedingly well presented.

Although there is a great diversity of experimental work included in this volume, the greater number of papers deal with spontaneous and induced mutations in fungi, bacteria, and viruses and with the use of such mutant strains in the elucidation of biochemical processes, pathogenic manifestations, etc., in the living cell (or unit). Recent developments in the study of genic control of biochemical processes in *Neurospora* are reported by W. D. BONNER and FRANCIS J. RYAN. Of particular interest is the rapidly accumulating mass of information on quite comparable phenomena in bacteria and viruses which would appear to be due to genic control. Papers by E. L. TATUM, JOSHUA LEDERBERG and E. L. TATUM, RENÉ J. DUBOS, MARY I. BUNTING, M. DEMEREC and R. LATARJET, S. E. LURIA, and ANDRÉ LWOFF emphasize the similarity, if not identity, of fundamental and intermediate control of synthetic processes, metabolic degradations, etc., in bacteria and in the higher fungi, where, because of the operation of a sexual mechanism, these activities can be shown to be under genic control. Viral mutations, originating spontaneously or as a result of irradiation, affecting various characteristic activities of viruses such as pathogenicity, lysis ability, plaque form, etc., and indicating a similar apparent controlling mechanism, are described in papers by THOMAS F. ANDERSON, M. DELBRUCK and W. T. BAILEY, JR., A. D. HERSHEY, and N. W. PRIE.

Three of the papers dealing with mutations in bacteria and viruses—those by HERSHEY, LEDERBERG and TATUM, and LURIA—present evidence that in these lowest organisms there exists a mechanism which accomplishes recombination and segregation of genetic factors, the results of which are similar to those of the Mendelian process in higher forms. Along quite different lines a paper by G. PONTECOVO deals with the applicability and the necessary modifications of classical genetical analysis and methods in the study of heterocaryotic systems in the higher fungi and particularly in the Fungi Imperfecti—i.e., in all forms lacking sexual reproduction but commonly showing hyphal fusions and intimate nuclear associations. Thus it would appear that the last frontiers are falling before the expanding utility of genetical techniques in the elucidation of fundamental processes.

The mechanism by which the gene controls the elaboration, duplication, and activity of complex cytoplasmic constituents is the essential feature of papers by S. SPIEGELMAN and CARL C. and GERTRUDE LINDEGREN. In both of these papers the be-

havior of certain self-duplicating yeast characters, which at meiosis do not follow random segregation, in respect to genic constitution and environmental factors constitutes the bases for hypotheses concerning the intermediate steps of genic action on the ultimate activities of the cell. A paper by T. M. SONNEBORN describes the experimental means by which the quantity of a similar (?) self-duplicating cytoplasmic factor (kappa of the gene K-kappa-killer substance complex) can be altered and also the significance of the gene-cytoplasmic factor relationship. The production of distinctive constituents by certain tumor cells is described by JOHN G. KIDD, and the gene-plastid relationship and the evidence for plastid mutations in higher plants are discussed by M. M. RHOADES.

The symposium also includes a number of papers of particular interest dealing with the general subject of heredity and variation in micro-organisms which pertain to matters other than genic mutations and gene-cytoplasmic constituent relationships. Among these are discussions of the following subjects: the need for, and difficulties attending the development of, a natural system of classification of the bacteria, by C. B. VAN NIEL; variations and inheritance in rust fungi, by T. JOHNSON; and complex reproductive processes in bacteria under certain conditions of admixture, by L. DIENES.

A most welcome addition to the symposium, while not contributing directly to the chosen topic of discussion, is a very understandable description of the theory and practice of phase microscopy.

While this volume will be read, in its entirety or in part, with much interest by biologists generally, it must surely be considered required reading for the students of two large fields of biological endeavor: genetics and microbiology.—JOHN R. RAPER.

An Introduction to Plant Anatomy. By ARTHUR J. EAMES and LAURENCE H. MACDANIELS. 2d ed. New York: McGraw-Hill Book Co., 1947. Pp. xvii+427. Illus. \$4.50.

The viewpoint and aims have not changed in this new edition of a standard text. The gross organization of the book has remained the same as in the first edition except that the chapter on the history of plant anatomy has been removed to allow for inclusion of additional descriptive material in the other chapters. Reorganization of the individual chapters varies from little, where essentially no new knowledge has recently come to light, to considerable in those chapters dealing with subject matter which has received much additional study during the last twenty years.

Chapters which have undergone most extensive "modernization" are those on the cell, meristems, tissues and tissue systems, periderm and abscission, leaf and flower, fruit and seed.

In the chapter on the cell a brief discussion of intrusive and symplastic growth is found in addition to that on gliding growth. The material on wall structure has been greatly enlarged in the light of extensive research during the last two decades.

The classification of meristems has been revised, and a discussion of the three main theories of structural development and differentiation—apical cell, histogen, and tunica-corpus—included. Descriptions of stem (vegetative), root, and floral apices, pointing out similarities and differences among them, should be helpful to the student.

The discussion of phloem anatomy has been brought up to date, taking into account the greatly increased knowledge in this area.

Considerably more is now known about the details of abscission—the formation, or lack of formation, of separation layers, periderms, etc.—than was known at the time of the first edition; this information is included particularly in the relatively detailed discussion of specific forms: *Castanea*, *Catalpa*, *Betula*, and *Populus*.

Revision in the chapter on the leaf deals particularly with leaf ontogeny. Discussions of the development of specific leaf tissues from the various meristematic regions and the duration of activity of such meristematic cells are useful additions. In this chapter a new three-dimensional cellular drawing of a typical leaf has been added and should prove a great help to both teacher and student.

Discussion of the vascular anatomy of the flower has been greatly enlarged and brought up to date. It is as comprehensive as such a discussion could be in an elementary text and should even be helpful as a starting-point for more advanced work. The reviewer personally regrets the use of the terms "fusion," "union," "cohesion," and "adnation" throughout this section to refer not only to cases in which actual fusion of initially independent parts does occur in ontogeny but also to those cases in which primordia are never independent and are completely non-diverged from their initiation.

The illustrative material has been increased, particularly in those areas showing the greatest revision. Many of the new figures are excellent photomicrographs used in conjunction with old and new line drawings.

The list of references at the end of each chapter has been brought up to date and considerably enlarged, again in those areas in which most revision has been done.

No attempt has been made in this review to report on the book as a whole, since it is assumed that those interested are familiar with the great usefulness of the original edition and are acquainted with the authors' clear presentation. A summary of changes made in this new edition should therefore merely point out the increased value of this already favorite text.—BARBARA F. PALSER.

FURTHER INVESTIGATIONS ON THE RELATION OF PHOTO PERIOD TO THE BORON REQUIREMENT OF PLANTS¹

B. ESTHER STRUCKMEYER AND ROBERT MACVICAR

Introduction

It was shown in a previous report (2) that plants of soybean (vars. Manchu no. 3 and Biloxi) exhibited a diminished requirement for boron when grown in a short-day environment. Plants grown in a boron-deficient culture medium and maintained in the vegetative state by a lengthened photoperiod showed morphological and anatomical abnormalities. In short photoperiods which induced blossom formation, however, the plants made essentially normal growth. It was found that the boron content of the tissues of plants grown in short day with and without boron was essentially identical. This limited manifestation of boron-deficiency symptoms in a short photoperiod has also been reported by SKOK (4) and by WARINGTON (8).

The purpose of the following experiments was to investigate further this condition with species that respond to photoperiod and with those that are day-neutral. The effects of relatively short induction periods on the response to boron deprivation were also investigated.

Material and methods

The following species of plants were used: cocklebur (*Xanthium echinatum* Murr.), buckwheat (*Fagopyrum esculentum* Gaertn.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill., var. John Baer), and soybean (*Glycine max* Merr., vars. Biloxi and Pagoda). They were grown under condi-

tions similar to those previously described (2). Seeds were germinated in either soil or silica sand, and seedlings were transferred to washed silica sand in varnished 10-inch clay pots. Approximately 500 ml. of the following nutrient solution were added on alternate days.

K ₂ HPO ₄	0.0012 Molar
KH ₂ PO ₄0018
CaSO ₄0021
CaCl ₂0010
MgSO ₄0021
NH ₄ NO ₃	0.0063

Minor elements and iron were supplied at the levels recommended by HOAGLAND and ARNON (1). Pots were leached at weekly intervals to remove unbalanced solution.

The following greenhouse conditions were maintained. During the winter months the light intensity rarely exceeded 750 foot-candles. Short photoperiod was obtained by using manually operated screens. Incandescent light bulbs of 40-watt capacity were used to extend the photoperiod from near sunset to midnight, providing daylengths of approximately 9 and 16 hours, respectively.

The sampling and analytical procedures have been previously described (2, 3). Samples for microscopic examination were taken at the fourth internode from the apex. A formalin-aceto-alcohol fixative was used; the fixed samples were dehydrated in *n*-butanol and imbedded in paraffin. Stem samples cut 12 μ in thickness were stained with iron-alum-hematoxylin and safranin.

¹ Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

Observations

EXPERIMENT I.—Young seedlings of tomato, buckwheat, sunflower, and cocklebur were transplanted from soil or sand to washed sand. Cocklebur and tomato plants were transferred to both short and long photoperiods on February 23, 1946; buckwheat and sunflower on March 2, 1946. In both daylengths, reduction in growth rate in minus-boron cultures was apparent after 12 days for tomato and 5 days for sunflower. No external differences were apparent in buckwheat or cocklebur at this time. By March 19, severe symptoms of boron deficiency had appeared in all tomato and sunflower plants without boron, regardless of photoperiod. Buckwheat plants deprived of boron were smaller than the controls, but no external symptoms of deficiency were evident; they were in fruit regardless of daylength or boron supply. Cocklebur plants grown on short photoperiod were fruiting. Symptoms of boron deficiency could not be noted in plants on short day except for slightly reduced growth, but on long photoperiods the vegetative plants were showing typical symptoms of deficiency, such as death of the stem tip, abnormal development of leaf and root, and fragility of petioles. All these external symptoms of pathology were lacking in cocklebur plants on short photoperiod. These relative conditions were essentially the same at the time of final harvest on April 8, 1946. By this time, tomato and sunflower showed severe symptoms of boron deficiency in both daylengths. Plants of buckwheat without adequate boron were small in size but otherwise appeared normal. Seeds were produced in both photoperiods, with and without boron. Cocklebur grown without boron continued to exhibit normal growth and development in short days and severe symptoms of deficiency in long

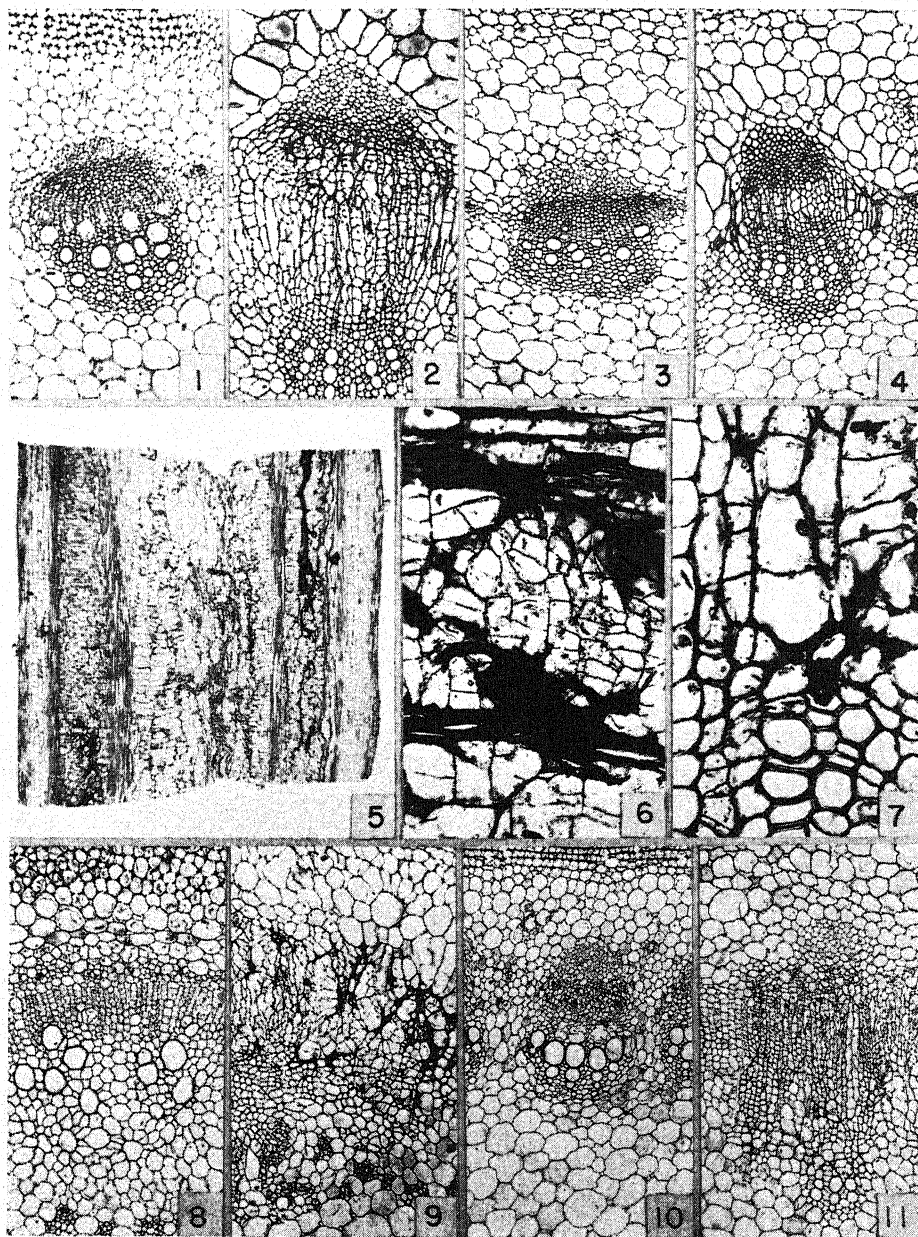
days. Data on the dry weight of plants, percentage of dry matter, top-root ratio, ash, and boron content are given in table 1.

Cocklebur is strongly sensitive to photoperiod, remaining vegetative in daylengths of 16 hours. The fourth internode from the stem tip of these plants grown with adequate boron showed the anatomical characteristics of a vegetative stem, such as an active cambium, the differentiation of vascular tissue, and relatively thin-walled cells (5) (fig. 1). Vegetative plants deprived of boron developed a pathological structure in that the fourth internode of plants exposed to 24 long days was distinctly abnormal; after 44 days of such treatment the abnormalities were much more pronounced. Some of the cells in the cambial region of the abnormal stems were enlarging radially and others were proliferating (fig. 2). The latter is especially convincing from longitudinal sections in which the cells of the cambial region show prominent nuclei and have divided irregularly and without regard to plane of division (fig. 5). Among the abnormal thin-walled parenchyma cells were rows of phloem and xylem differentiating from still functional cambial cells. The sieve tubes and companion cells did not appear normal, and the xylem elements formed were frequently of the scalariform and reticulate types. The ray cells of the interfascicular region enlarged radially. Proliferating cells were apparent in the cortex and pith, and, in the more severely affected areas, necrosis was evident. Necrosis was apparent principally in the vascular bundle (figs. 2, 6). These darkened necrotic regions were caused in part by collapsed cells resulting from disturbed growth of the enlarging cambial zone. The sieve tubes and companion cells and tracheal elements formed from the abnormal cambium also seemed frequently

TABLE 1

EXPERIMENT I. EFFECT OF PHOTOPERIOD ON GROWTH AND BORON CONTENT OF
VARIOUS PLANTS SENSITIVE AND INSENSITIVE TO PHOTOPERIOD

PLANT AND TREATMENT	TOTAL DRY WEIGHT OF LEAF AND STEM (GM.)	DRY MATTER (%)	TOP/ROOT RATIO	LEAF AND STEM TISSUE	
				Ash (%)	Boron (p.p.m.)
3-19-46 sampling					
Sunflower, long+B.....	0.98	6.2	2.1		
Sunflower, long-B.....	0.57	6.8	3.8		
Sunflower, short+B.....	0.92	5.5	5.7		
Sunflower, short-B.....	0.54	6.0	5.4		
Buckwheat, long+B.....	0.25	4.5			
Buckwheat, long-B.....	0.20	5.0			
Buckwheat, short+B.....	0.40	6.3			
Buckwheat, short-B.....	0.17	5.0			
Tomato, long+B.....	2.60	9.0	3.7	24.5	90.5
Tomato, long-B.....	1.40	8.4	7.4	20.9	8.9
Tomato, short+B.....	1.85	5.9	3.7	25.4	118.7
Tomato, short-B.....	1.10	7.4	3.7	21.5	10.9
Cocklebur, long+B.....	2.20	8.6	4.8	17.5	110.0
Cocklebur, long-B.....	1.55	9.4	9.7	16.0	20.0
Cocklebur, short+B.....	1.10	8.5	8.4	18.9	129.2
Cocklebur, short-B.....	1.05	8.9	10.5	21.2	18.8
4-8-46 sampling					
Sunflower, long+B.....	9.25	10.9	3.7	14.0	51.9
Sunflower, long-B.....	2.10	7.5	4.0	22.9	8.2
Sunflower, short+B.....	4.25	9.1	3.9	13.4	62.0
Sunflower, short-B.....	1.10	8.4	2.8	15.7	12.3
Buckwheat, long+B.....	1.85	11.5	6.1	11.6	45.0
Buckwheat, long-B.....	0.55	11.0	4.2	13.9	12.7
Buckwheat, short+B.....	1.35	16.2	6.7	13.3	41.6
Buckwheat, short-B.....	0.55	12.5	4.6	13.6	14.7
Tomato, long+B.....	9.80	10.8	4.9	14.1	69.4
Tomato, long-B.....	1.30	7.7	4.8	24.3	9.1
Tomato, short+B.....	9.00	9.1	5.3	16.6	58.0
Tomato, short-B.....	1.05	7.2	5.2	28.0	9.0
Cocklebur, long+B.....	8.30	12.5	2.8	10.9	86.5
Cocklebur, long-B.....	2.20	11.9	4.4	14.7	11.4
Cocklebur, short+B.....	3.25	11.4	5.2	14.5	115.8
Cocklebur, short-B.....	2.75	11.6	5.3	15.7	10.6



FIGS. 1-11.—Figs. 1-4, cross sections of fourth internodes of cocklebur. Fig. 1, normal structure in vegetative plant grown with boron in long photoperiod. Fig. 2, abnormal structure in plant grown without boron in long photoperiod; cells of cambial region enlarged and increased in number; necrotic areas in region of secondary phloem. Fig. 3, normal structure in fruiting plant grown with boron and short photoperiod. Fig. 4, slightly abnormal structure in plant grown without boron and in short photoperiod; only two bundles in stem showed symptoms of boron deficiency. Figs. 5-7, longitudinal and cross sections of fourth internodes of cocklebur plants grown without boron in long photoperiod. Fig. 5, extent of cambial proliferation apparent; extensive necrosis and disorganization of vascular tissue; abnormal and crushed cells in pith. Fig. 6, enlargement of area in figure 5 showing detail of necrotic regions; part of necrosis result of collapsed and crushed cell walls. Fig. 7, incipient collapse of cell walls in xylem region; distortion and partial collapse of cell wall of xylem element result of increase in size and proliferation of adjoining cells. Figs. 8-11, cross sections of fourth internodes from tomato and sunflower plants. Fig. 8, normal structure in blossoming tomato grown with boron in long photoperiod. Fig. 9, abnormal structure in tomato grown without boron in long photoperiod; proliferation of cells in cambial region and extensive necrosis. Fig. 10, normal structure of vegetative sunflower grown with boron in short photoperiod. Fig. 11, abnormal structure of sunflower grown without boron in short photoperiod; presence of abnormal cambial cells, proliferation, and necrosis evident.

to be a part of this necrosis (fig. 7). WARINGTON (7) has observed this darkening in stems of carrot and has described it as the disintegration of the cambium. Some bundles of the abnormal stem showed more cellular disorganization than others. Cell division occurred frequently in the region of the perimedullary zone. Plants exposed to long days remained vegetative; the cambial zone in the stems was from six to eight cells wide, and these cells were the most sensitive to a minus-boron treatment.

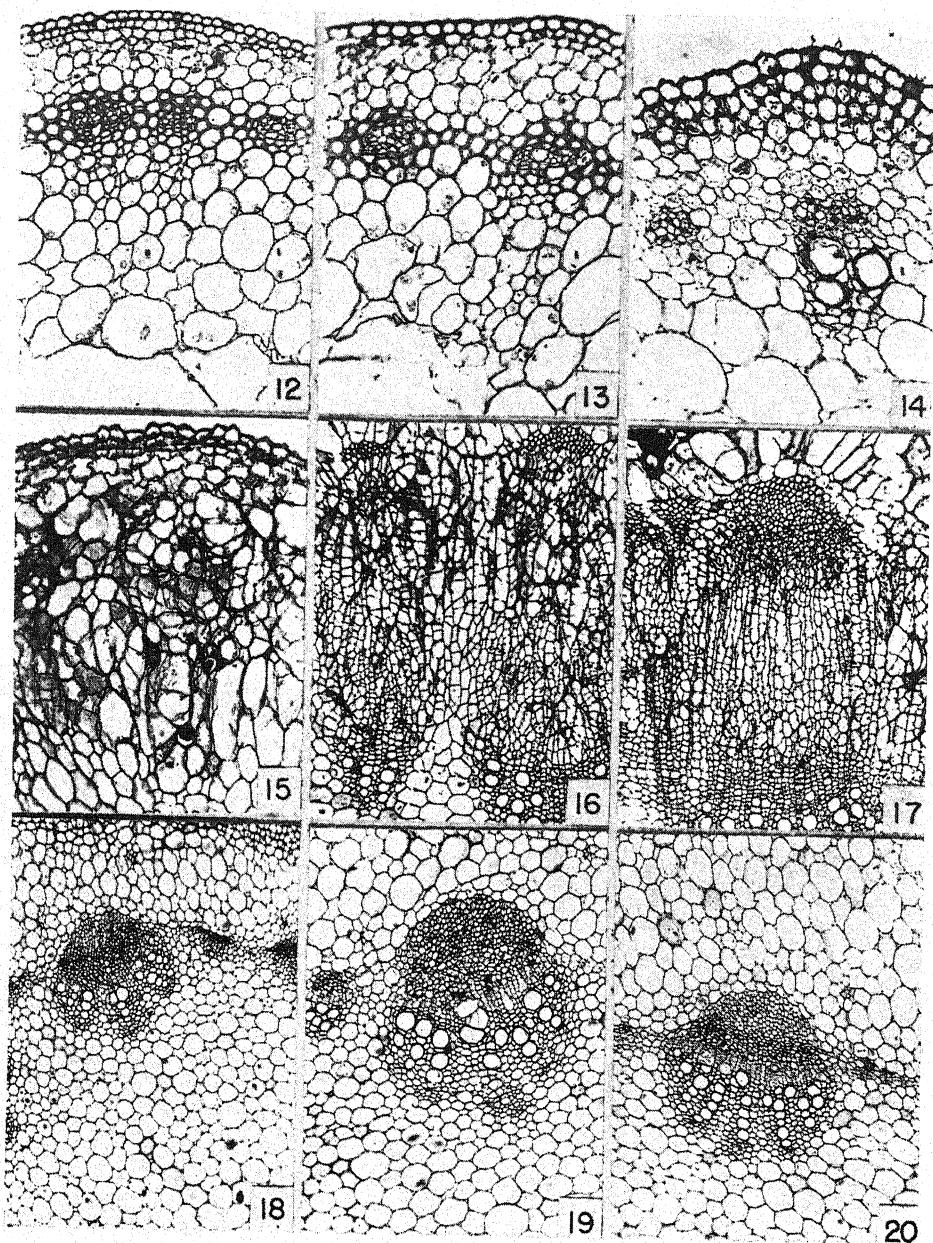
Cocklebur forms fruits in short days and acquires the characteristics of a fruiting stem (fig. 3). These characteristics were apparent after exposure to 24 short days with boron; after 44 days the plants remained unchanged. Symptoms of boron deficiency were not apparent in the structure of the stems of plants exposed to 24 short days and grown without boron. Only two vascular bundles in the stems of plants given 44 short days without adequate boron gave indication of cellular abnormality. The cells of the cambial zone and the medullary ray had started to enlarge. Necrotic regions were not apparent (fig. 4).

Tomato, sunflower, and buckwheat are day-neutral and do not make specific responses to photoperiod. Tomato grown with boron and exposed to short or long photoperiods for 44 days was in blossom and displayed a normal structure for a blossoming stem (fig. 8). Tomato plants grown without boron and exposed to short or long days showed extreme cellular disorganization of the stem, the three main bundles being most severely affected. The structure of the stem was primarily the result of abnormal cambial activity, as has been described for cocklebur. Cells of the cambial zone enlarged, and some of them proliferated. This resulted in a disrupted region of tissue,

some of which was crushed, thereby forming strands of necrotic areas. There also appeared to be some proliferation of the internal phloem. The cortex and pith remained normal at this stage (fig. 9).

Sunflower had no macroscopic flowers at either sampling, and the plus-boron plants after 44 short days showed a vegetative anatomy in the stem (fig. 10). Symptoms of boron deficiency in the structure of the stem were similar in type to those previously described for cocklebur and tomato. In this species, also, the cambium exhibited the same type of abnormal cell extension and proliferation (fig. 11). The degree of severity was similar in the plants grown without boron in both daylengths.

Buckwheat is different from the other test plants in the extreme rapidity with which it comes to flower. By the twelfth day following transplanting, macroscopic flower buds had appeared. Plants grown with boron and exposed to 24 short days exhibited the type of stem anatomy characteristic of the fruiting plant (fig. 12). Plants grown without boron in short photoperiods showed no external abnormalities other than restricted growth; no evidence of abnormality was observed in the anatomy of the stem after 44 short days (fig. 13). In long days the stems of plants grown with boron showed a less mature development of cells, indicative of a more recent cessation of cambial activity. The vascular elements had not fully matured, and thin-walled elements were still present (fig. 14). Plants grown in long photoperiods and in boron-deficient cultures showed evidence of internal abnormality, in that the cambial area was again the zone of cellular disorganization. Some of the cells were enlarging and others were dividing, resulting in a very distorted pattern of the vascular system (fig. 15).



FIGS. 12-20.—Figs. 12-15, cross sections of fourth internodes of buckwheat. Fig. 12, normal structure in flowering plant grown with boron in short photoperiod. Fig. 13, structure in flowering plant grown without boron in short photoperiod; typical flowering anatomy; no proliferation or necrosis apparent. Fig. 14, grown with boron in long photoperiod; plants in flower, but anatomical structure shows evidence of more recent cambial activity as compared with figure 12. Fig. 15, abnormal structure in plant grown without boron in long photoperiod; proliferation and disorganization of vascular tissue evident; necrosis also apparent. Figs. 16-20, cross sections of fourth internodes of cocklebur; some plants exposed to short and long days continuously; others given short-day induction periods of varying lengths and returned to long days. Fig. 16, grown without boron, given 4 days of induction and then transferred to long days; cellular disorganization and necrosis apparent. Fig. 17, grown continuously in long days without boron; abnormal cambial activity and necrosis apparent. Fig. 18, grown without boron in short days. No evidence of cellular disorganization; normal fruiting anatomy. Fig. 19, grown with boron, given 10-day induction period, and returned to long days; normal structure apparent. Fig. 20, grown without boron, given 10 short days, and returned to long days; no evidence of proliferation or other cellular disturbances.

EXPERIMENT II.—This experiment was similar to experiment I, but only cocklebur and tomato were used as test plants. Cocklebur plants were placed in differential photoperiod on March 23, 1946, and harvested approximately 8 weeks later; tomato plants were transferred June 5 and harvested 7 weeks later. Cocklebur plants grown in short

was induced by exposure to a few short days rather than numerous short photoperiods was studied in cocklebur. Plants were transferred to both short and long photoperiods; one group was given an induction of 4 short days and then transferred to the long-day environment. Boron-deficiency symptoms appeared shortly after the return of the induced

TABLE 2
EXPERIMENT II. EFFECT OF PHOTOPERIOD ON GROWTH AND BORON CONTENT
OF NORMAL AND BORON-DEFICIENT TOMATO AND COCKLEBUR PLANTS

PLANT AND TREATMENT	TOTAL DRY WEIGHT OF LEAF AND STEM (GM.)	DRY MATTER (%)	TOP/ROOT RATIO	LEAF AND STEM TISSUE	
				Ash (%)	Boron (p.p.m.)
	4-20-46 sampling				
Cocklebur, long+B.....	16.75	14.6	3.0	13.2	71.0
Cocklebur, long-B.....	9.65	14.1	3.4	13.0	18.8
Cocklebur, short+B.....	13.55	13.9	5.4	12.4	89.6
Cocklebur, short-B.....	7.80	12.1	6.2	15.1	20.0
	7-24-46 sampling				
Tomato, long+B.....	10.60	10.0	2.5	11.4	66.4
Tomato, long-B.....	3.60	12.6	7.2	28.4	12.8
Tomato, short+B.....	11.40	10.5	3.0	14.9	73.7
Tomato, short-B.....	3.20	9.6	6.4	25.1	14.6

day without boron made essentially normal growth, although the number of flower buds was limited and the plants were distinctly smaller than the plus-boron controls. In long photoperiod, however, severe deficiency symptoms developed in the absence of boron. The symptoms of deficiency in tomato were similar in short and long days. Histological findings for cocklebur and tomato were the same as those noted in experiment I. Data on the weights of the plants, etc., are presented in table 2.

EXPERIMENT III.—The effect on the boron requirement when floral initiation

minus-boron plants to long photoperiods. Ten days after the induction period, the first blossom buds had become apparent in the induced plants receiving adequate boron. As is usually the case, however, with plants exposed to such short induction periods, the plants continued to grow, and blossom development was slow. Symptoms of boron deficiency were present both in those plants given an induction period of 4 short days and in those grown continually in long days in the absence of this element. The plants receiving the 4-day induction were somewhat larger than those kept continuously

in short days. The internal structure of the stems of induced plants grown without boron showed severe disturbance in the cambial region. As previously described for cocklebur, some of the cambial cells ceased differentiation and became enlarged; others continued to divide, but the derivatives did not differentiate into phloem elements and remained as meristematic or parenchymatous cells. The ray cells also showed this phenomenon. Advanced stages of necrosis were also evident (fig. 16). The plants grown without boron in long days continuously showed the same type of disorganization of the cells as those given 4 short days (fig. 17).

Since the 4-day induction period did not prevent the development of boron-deficiency symptoms, a second test of the effect of induction on the expression of such symptoms was made. Cocklebur plants were grown with adequate boron. After 1 week half of the pots were thoroughly leached, and a minus-boron solution was supplied. One set of plants was given an induction period of 10 long nights, after which they were transferred to long days. Other groups were maintained continuously in long or short photoperiods. Those plants receiving 10 days of induction showed macroscopic flower buds 14 days after the beginning of the induction period. At the time of harvest, 3 weeks later, those plants receiving adequate boron and continuously on long photoperiod were normal vegetative plants; those plants deprived of boron on long days showed severe symptoms of deficiency. Complete necrosis of the apical meristem, malformation of leaf tissue, and fragility of the upper leaves and petioles were striking evidence of the lack of this element. In addition, a marked thickening of the stem in the region of the third and fourth internode

was noted. Plants given a 10-day induction period while receiving adequate boron were normal fruiting plants; minus-boron cultures had aborting blossoms and slight symptoms of deficiency. These symptoms were far less severe, however, than in those plants maintained in the vegetative state by continuous exposure to long days. Plants grown continuously in short day were fruiting without regard to the presence or absence of boron; minus-boron plants were showing restricted rates of growth. Data on the dry weights of plant parts, etc., are presented in table 3.

Histological examination of cocklebur plants grown in short days with and without boron showed a typical fruiting stem and no abnormalities with the absence of boron (fig. 18). Plants receiving the 10-day induction period with boron had the normal anatomy of a fruiting stem (fig. 19). Induced plants without boron were essentially similar. There was some evidence of incipient disorganization in the cortex, but the vascular tissue appeared normal, with no abnormal cambial activity (fig. 20). Those plants maintained in long photoperiod continuously and, having no boron, exhibited the usual abnormalities, including the enlargement of cells of the cambial region (fig. 20); those grown with adequate boron showed the vegetative type of stem anatomy.

EXPERIMENT IV.—In order to compare more directly the relation of flowering and the compensatory effect of shortened photoperiod with boron requirement, an additional test was made using two varieties of soybean, one a short-day type and the other day-neutral. One variety, Biloxi, remains vegetative in long photoperiod; the other, Pagoda, is insensitive to photoperiod, producing fruits over a wide range of daylengths. Week-old seedlings were transferred to

differential daylengths. Twenty-five days later, plants of Biloxi grown in long days and without boron showed typical signs of boron deprivation. These became progressively more severe. All other cultures remained without obvious outward symptoms until the time of harvest, 60 days after the start of the treatment. The

essential difference in the anatomical structure of plus-boron plants and those deprived of boron (figs. 22, 23, 24, 25). Biloxi, on the other hand, showed a slight indication of abnormality in short days without boron. Proliferation of cells in the cambial zone appeared to have occurred, but there was no evidence of

TABLE 3
EXPERIMENT III. EFFECT OF PHOTOPERIODIC INDUCTION ON GROWTH AND BORON
CONTENT OF NORMAL AND BORON-DEFICIENT COCKLEBUR PLANTS

PLANT AND TREATMENT	TOTAL DRY WEIGHT OF LEAF AND STEM (GM.)	DRY MATTER (%)	TOP/ROOT RATIO	LEAF AND STEM TISSUE		
				Ash (%)	Boron (p.p.m.)	
5-6-46 sampling						
	Continuous long+B.	13.95	10.9	3.2	13.9	86.4
	Continuous long-B.	11.50	12.2	4.0	11.1	23.0
	10-day induction+B.	14.30	11.6	5.7	13.8	85.6
	10-day induction-B.	12.50	12.0	5.3	12.4	25.0
	Continuous short+B.	11.65	12.4	5.1	13.9	66.0
	Continuous short-B.	7.80	10.4	4.1	14.9	26.3
	5-15-46 sampling					
	Continuous long+B.	29.35	14.4	3.9	11.3	68.1
	Continuous long-B.	14.00	16.7	4.3	13.0	19.1
	4-day induction+B.	31.15	15.1	4.3	10.5	72.5
	4-day induction-B.	20.60	16.5	5.0	12.8	21.1

Pagoda variety grown without adequate boron was reduced in size, but the appearance of leaves, stems, fruits, and roots was not dissimilar to that of plants receiving adequate quantities of this element. Data on tissue weights, etc., are given in table 4.

Histological examination of the fourth internode gave findings closely correlated with the above. Pagoda exhibited the anatomical features typical of fruiting stems under all treatments. There was no

necrosis or other serious abnormalities (fig. 27). Plants grown on short days with boron showed the anatomical structure typical of a fruiting stem (fig. 26). In long days, Biloxi retained a stem structure indicative of its vegetative condition (fig. 28), while in Pagoda, which was fruiting, cambial activity had ceased, and there was a general thickening of the cell walls (fig. 24). In accordance with previous findings in Biloxi (2), boron deprivation on long photoperiod resulted in

proliferation in the cambial zone with accompanying necrosis, particularly in the secondary phloem (fig. 29).

Discussion

The results obtained in these and previous experiments indicate that abnormal cambial activity is a common characteristic of boron deficiency in the plants studied. Correlation of anatomical observations with external morphology

buckwheat, and soybean—in which the cambium becomes inactive at an early stage, the symptoms of boron deficiency are repressed or absent. Photoperiodic induction of flowering, resulting in the maturation of the cambium, leads to limited external and internal pathology in Biloxi soybean and cocklebur deprived of boron. When photoperiodic induction is insufficient to halt cambial activity, symptoms of boron deficiency appear.

TABLE 4
EXPERIMENT IV. EFFECT OF PHOTOPERIOD ON GROWTH AND BORON CONTENT OF SENSITIVE AND INSENSITIVE SOYBEAN PLANTS WITH AND WITHOUT ADEQUATE BORON

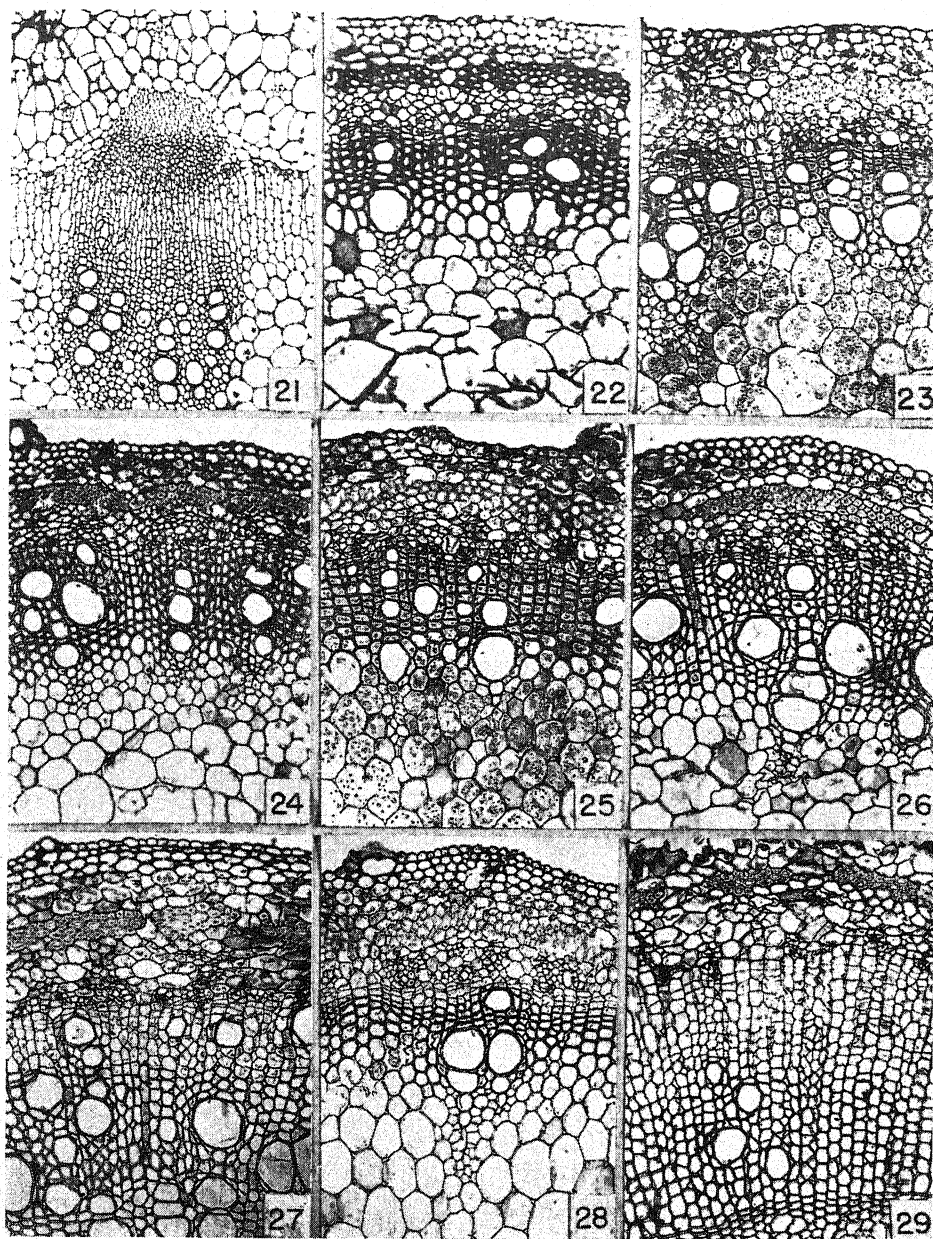
PLANT AND TREATMENT	TOTAL DRY WEIGHT OF LEAF AND STEM (GM.)	DRY MATTER (%)	TOP/ROOT RATIO	LEAF AND STEM TISSUE	
				Ash (%)	Boron (p.p.m.)
Pagoda, long+B.....	13.85	24.5	10.6	7.1	46.5
Pagoda, long-B.....	8.90	28.6	8.9	7.6	10.7
Pagoda, short+B.....	8.90	24.6	9.9	9.5	58.6
Pagoda, short-B.....	7.70	25.6	9.1	9.1	13.8
Biloxi, long+B.....	12.50	24.7	3.1	7.3	54.6
Biloxi, long-B.....	6.60	23.4	4.4	10.5	11.0
Biloxi, short+B.....	10.75	19.7	3.9	9.3	56.3
Biloxi, short-B.....	8.15	19.8	5.8	10.9	15.2

reveals that internal abnormalities are apparent when external symptoms only are slightly evident and sometimes lacking. Most previous investigators of the histopathology of boron deficiency have noted, but not adequately emphasized, this abnormal growth and proliferation in the cambial zone. The specific lesions resulting from collapsing cells are usually emphasized, while the abnormal cambial activity producing them has received scant attention. WALKER (6), however, recognized the significance of this activity and attributed to it many of the external symptoms of deficiency.

In those plants—such as cocklebur,

When longer periods of induction are employed, the cambium becomes less active and symptoms of boron deficiency are not apparent. Soybean var. Pagoda and buckwheat fall into a group which initiates floral primordia very early in development. In these species absence of boron restricts the absolute amount of growth, but the typical pathology usually associated with boron deficiency is reduced in intensity or absent.

That an increased boron content in the tissue is not the cause of this diminution in symptomatology can be concluded from the data on boron content of the tissue. In each case plants showing symptoms of



FIGS. 21-29.—Fig. 21, cross section of stem of cocklebur grown without boron continuously in long days; extensive proliferation of cambial region and necrosis apparent. Figs. 22-25, cross sections of fourth internodes of fruiting soybean, var. Pagoda. Fig. 22, grown with boron in short days; almost complete cessation of cambial activity. Fig. 23, grown without boron in short photoperiod; structure of stem normal with no indication of abnormal cambial activity. Fig. 24, grown with boron in long days; anatomical structure typical of fruiting stem. Fig. 25, grown without boron in long photoperiod; stem shows fruiting anatomy and no cellular abnormalities. Figs. 26-29, cross sections of fourth internodes of soybean, var. Biloxi, which flowers only in short days. Fig. 26, grown with boron in short days; stem shows flowering structure. Fig. 27, grown without boron in short photoperiod; slight proliferation of cells in vascular region apparent; no indication of necrosis. Fig. 28, grown with boron in long photoperiod; active cambium typical of vegetative stem. Fig. 29, grown without boron in long days. Anatomical structure typical of vegetative stem with boron-deficiency symptoms apparent; proliferation and enlargement of cells in cambial region; some evidence of necrosis in region of phloem.

deficiency and those without symptoms have the same amount of boron. The boron content of plants grown in long days without boron, which show severe deficiency symptoms, is not different from that in plants grown in short days without boron which show no symptoms. This is in accordance with previous observations on Biloxi and Manchu soybeans.

Summary

1. The reduction of intensity of boron-deficiency symptoms, associated with blossoming induced by short photoperiod, has been confirmed and extended.

2. Cocklebur plants grown without boron and exposed to short days fruited and showed no deficiency symptoms. Plants grown in long days without boron remained vegetative and soon displayed both external and internal signs of boron deficiency. This diminution of symptoms in short days was not the result of an increased boron absorption.

3. Continuous exposure to short days is not required for floral initiation in cocklebur, provided an induction period of suitable length is given the plants. Plants grown without boron and given 4 short days, after which they were exposed to long days, showed severe symptoms of boron deficiency; however, plants given an induction period of 10 short days and then returned to long days gave no evidence of boron deficiency.

4. Cocklebur plants exposed to 10 short days displayed reduced cambial activity which was associated with flowering. Boron deprivation in the stem caused tissue disorganization resulting from abnormal activity of the cambium; the cambium in the plants given 10 short days became less active, so that, after return to long days, proliferation and enlargement of cells in the cambial zone, resulting from minus-boron treatment, did not occur.

5. Tomato, sunflower, and buckwheat are day-neutral plants. With the exception of buckwheat, these plants flower after a considerably greater length of time than cocklebur. Plants of tomato and sunflower grown without boron and regardless of length of day showed striking symptoms of boron deprivation. The external symptoms included the typical death of the stem tip, abnormal development of leaves and roots, and fragility of the petioles. The internal structure of the stem was similar to cocklebur in that abnormal cambial activity occurred, resulting in proliferation and enlargement of cells. In plants showing severe symptoms of boron deficiency, necrotic areas were conspicuous. Buckwheat flowered at an early stage. In short days cambial activity ceased sooner than it did in long days. Symptoms of boron deficiency were not apparent in short days, even in those plants grown without boron. In long days, however, maturation of tissues was less rapid, and slight symptoms of boron deficiency were apparent in plants grown without this element.

6. Soybean var. Biloxi is a short-day plant and resembles cocklebur in its response to boron deprivation. This variety fruited in short days and showed no symptoms when grown without boron. In long days, however, severe symptoms in minus-boron cultures were apparent. Pagoda soybean fruited at an early stage in both short and long days; deficiency symptoms were not evident in the minus-boron cultures on either photoperiod.

7. It is suggested that the decrease in cambial activity associated with early floral induction or photoperiodic induction causes a reduction in the severity of boron-deficiency symptoms.

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A STUDY OF THE ANEMOPHILOUS PLANTS OF PUERTO RICO

EDWARD P. CLAUS

Introduction

Although the flora of Puerto Rico, the largest Caribbean island possession of the United States, has been studied thoroughly (2, 4, 11, 13, 23, 24, 47), the allergenic plants have been neglected. There are no publications of allergy clinics such as those in the United States, Mexico, Brazil, and Argentina. The St. Luke's Memorial Hospital in the city of Ponce maintains the only allergy clinic in Puerto Rico known to the writer.

During a year's residency in 1944-45 the writer saw many persons suffering from asthma, sinusitis, and other allergic symptoms. More recently, in an effort to ascertain whether wind-borne pollen grains were abundant in the atmosphere, he sent filmed slides to the island. These were exposed¹ in the manner described by WODEHOUSE (53). Microscopic examinations of these slides have revealed the presence of pollen grains, mold spores, ticks, starch grains, and other aerobiological matter. There were relatively more pollen grains than mold

spores in the atmosphere of the city of Río Piedras during the period February 8-17, 1946. This disagrees with the comments on allergy in Puerto Rico by WODEHOUSE (54). A daily pollen count at various locations on the island should be undertaken for a period of several years to substantiate the author's observation.

Dr. QUINTERO FOSSAS of Cuba, in an address before the Medical Association of Puerto Rico in 1941, stressed the need for investigations of air-borne pollens, mold spores, dusts, and other sensitogens. Previously (6) he had suggested the necessity of observing the spore content of the air in Cuba and the other West Indies islands. An anonymous article on hay fever in Latin America (1) indicates the lack of published data and literature on the subject. VAUGHAN (51) stated that further attention should be devoted to the problem of hay fever in the Americas. The address of QUINTERO FOSSAS (39) and a recent publication of the writer (10) indicate some of the plants which may cause pollen allergy in Puerto Rico.

¹ Exposed by Mr. ESTEBAN NÚÑEZ MELÉNDEZ, instructor in pharmacy, University of Puerto Rico College of Pharmacy.

THOMMEN (48) formulated the following five postulates that all allergist-botanists recognize:

1. The pollen must contain an excitant of hay fever.
2. The pollen must be anemophilous, or wind-borne, as regards its mode of pollination.
3. The pollen must be produced in sufficiently large quantities.
4. The pollen must be sufficiently buoyant to be carried considerable distances.
5. The plant producing the pollen must be widely and abundantly distributed.

According to these postulates the hay-fever plants of the United States are well determined. Some of these plants grow in Puerto Rico; thus, they may be regarded as causative agents in pollen allergy. Similarly, some of the hay-fever plants of Mexico, Argentina, Brazil, Uruguay, Cuba, and Bermuda also grow in Puerto Rico and may be considered allergenic. In addition, personal observations of wind-pollinated plants and studies of the available scientific literature reveal that other plants are related botanically to those already established as allergenic. A number of these are endemic to the island and do not occur in the countries mentioned above, while others more completely answer THOMMEN's fifth postulate of distribution. Therefore, they may be important factors in pollen allergy. This investigation represents a comparative study of the anemophilous plants of Puerto Rico and the allergenic anemophilous plants of North, Central, and South America.

Discussion

In referring to the plants the following sequence is used: the English name (if any) is followed in parentheses by the Spanish name (if any) as listed by OTERO and TORO (36) and then by the scientific name. In the case of grasses the termi-

nology of HITCHCOCK (21, 22) is employed wherever possible. For other plants the nomenclature of BRITTON and WILSON (4) is used, supplemented by SMALL (46).

GRASSES.—A number of important allergenic grasses grow in Puerto Rico. Some of these are cultivated as forage crops, some occur as troublesome weeds, and others are weeds of waste places. In any instance grasses represent a definite factor in pollen allergy, since their flowering period extends over the entire year.

Bermuda grass (pepe ortiz, yerba Bermuda), *Cynodon dactylon* (L.) Pers., is widespread over the Western Hemisphere, being a primary cause of pollinosis in the United States (55), in Mexico (42), in Argentina (8), in Brazil (20), in Uruguay (3), and in Cuba (39). Undoubtedly it is of foremost importance in the pollen allergy of Puerto Rico.

The pollen of goose grass (yerba dulce, pata de gallina), *Eleusine indica* (L.) Gaertn., is listed (18) as a secondary contributor to the atmospheric pollen in the southeastern states. Goose grass grows commonly in Argentina (8).

Molasses grass (yerba melado, yara-gua), *Melinis minutiflora* Beauv., is reported to be the chief origin of grass pollens in the atmosphere of Rio de Janeiro (19, 29). The pollen is present on atmospheric slides exposed in other Brazilian cities: Belo Horizonte (30), Salvador (31), Juiz de F6ra (32), Campinas (33), Barbacena (34), and Varginha (35). Since the pollen is so abundant in the air of South America, this grass is certainly of potential importance in pollen allergy in Puerto Rico. Molasses grass is not naturalized in North America.

Johnson grass (yerba Johnson), *Sorghum halepense* (L.) Pers., which is widely distributed in the United States (18), in Mexico (43), in Argentina (8),

and in Brazil (49), and sorghum (millo), *Sorghum vulgare* Pers., cause hypersensitivity. However, Johnson grass, because of its larger distribution, is more prominent in hay-fever literature.

Sugar cane (caña de azúcar), *Saccharum officinarum* L., has long been suspected as a cause of allergy in Puerto Rico. Its flowering period extends from the latter part of October to the early part of January. QUINTERO FOSSAS (39) indicated that sugar cane is a factor in pollen allergy in Cuba; similarly, RODDIS (40) listed it among the allergenic plants of Hawaii. Although the pollen is too heavy to be wind-borne for long distances, it must be regarded as a prominent constituent in pollinosis because the plants are so extensively cultivated on the island.

Natal grass, *Tricholaene rosea* Nees, is one of the significant grasses of Miami (27), shedding pollen in every month except January and February. Crabgrass, *Digitaria sanguinalis* (L.) Dulac., widely distributed in the tropic and temperate climates, is considered of secondary importance in inducing hay fever because it does not produce much pollen. St. Augustine grass (grama blanca), *Stenotaphrum secundatum* (Walt.) Kuntze, flowers almost continually and should be regarded as essential in pollen allergy. It is listed among the minor hay-fever plants of the southeastern states (18). Barnyard grass (arrocillo), *Echinochloa crus-galli* (L.) Beauv., is of secondary importance in pollen allergy.

There are many endemic and naturalized grasses in Puerto Rico that represent genera reported in the hay-fever literature of other countries. Since it has been ascertained that the sensitizing principle of pollen grains is usually characteristic of all species of a genus, these grasses may be suspected in pollen allergy. In

referring to *Bouteloua*, WODEHOUSE (55) made the statement: "In hayfever studies distinction is seldom made between the different species." Consequently, the grama, *Bouteloua americana* (L.) Scribn., and the slender grama, *B. heterostega* (Trin.) Griffiths, are important.

The finger grasses, *Chloris ciliata* Swartz; (horquetilla), *C. radiata* (L.) Swartz; (horquetilla morada), *C. inflata* Link; and *C. petraera* Swartz may be of importance as allergenic plants, since they are related to the feather finger grass listed by WODEHOUSE (55) as a minor hay-fever plant of Arizona.

Smut grass (burillo), *Sporobolus poiiretii* (Roem. and Schult.) Hitchc., is an important hay-fever grass (18). The West Indian rush grass (matojo), *S. indicus* (L.) R. Br.; (matojo de playa), *S. virginicus* (L.) Kunth.; (matojo cubano), *S. cubensis* Hitchc.; and *S. pyramidatus* (Lam.) Hitchc., are related species and may be of local importance.

The genus *Paspalum* is represented in Puerto Rico by about twenty species, the most prominent being the bull grass (cortadero), *P. virgatum* L. A related species is abundant in New Orleans (38). Additional species are mentioned in the hay-fever literature of the United States (55) and of Argentina (8).

Knotroot bristle grass, *Setaria geniculata* (Lam.) Beauv., and *S. setosa* (Swartz) Beauv., are the most numerous of the seven species in Puerto Rico. These may be of some importance in hay fever, since they are related to the green bristle grass of southern United States (18).

Sorghastrum (millito), *Sorghastrum setosum* (Griseb.) Hitchc., is related to a mainland species (27). Broom grass or broom sedge, *Andropogon virginicus* L., is listed as an important pollen-producing plant of Miami (27). Other island

species are: all-beard grass (rabo de raton), *A. virgatus* Desv.; bushy beard grass (yerba barbuda), *A. glomeratus* (Walt.) B.S.P.; (matojillo), *A. leucostachyus* H.B.K.; and (rabo de gato), *A. bicornis* L. The latter is widely distributed in the West Indies. Species allied to the vetiver or khus-khus (pachuli), *Veliveria zizanoides* (L.) Nash, are reported in Argentina (8) and in the United States (18) but of minor importance in hay fever.

Guatemala grass (yerba de Guatemala), *Tripsacum laxum* Nash, is not found in the United States. Related species are listed in Argentina (7) as possible allergenic plants. *Uniola laxa* (L.) B.S.P. is related to the seaside oats, a minor pollen-producing plant of Miami (27).

The allergenic pollen of Indian corn (maiz), *Zea mays* L., is of local importance only, since it is not carried for long distances by the wind. Cultivated white oats (avena), *Avena sativa* L., is of minor importance as a source of atmospheric pollen.

The grasses that follow may be of significance, since they undoubtedly contribute much pollen to the air. At present none of them has been determined allergenic: giant reed or cowcane (guajana, caña de castilla), *Arundo donax* L.; common reed or wild cane (caña de indio), *Phragmites communis* Trin.; Guinea grass (yerba de Guinea), *Panicum maximum* Jacq.; Para grass (malojilla), *P. purpurascens* Raddi; napier grass (yerba elefante), *Pennisetum purpureum* Schumacher; carib grass (malojilla), *Eriochloa polystachya* H.B.K.; carpet grass, *Axonopus compressus* (Swartz) Beauv.; and (yerba dorada), *A. aureus* Beauv. The pollen of Guinea grass commonly occurs on atmospheric slides of Brazil (29, 30).

Redtop, orchard grass, June grass, timothy, sweet vernal grass, rye grass, and the other essential hay-fever grasses

of North America are not found on the island.

HERBACEOUS PLANTS.—The troublesome hay-fever families of North, Central, and South America—Polygonaceae, Chenopodiaceae, Amaranthaceae, Plantaginaceae, and Compositae—are all represented in the flora of Puerto Rico. In addition, there are a few species of secondary importance in the following: Typhaceae, Cyperaceae, and Urticaceae.

Polygonaceae.—*Rumex* and *Persicaria* are the only allergenic genera growing on the island. Curled dock (col agria, vina-grillo), *Rumex crispus* L., is widespread in the United States, where it is considered of importance in pollen allergy (18). It is also common in Mexico (44) and in Argentina (8). The four species of *Persicaria*: *P. punctata* (Ell.) Small, *P. acuminata* (H.B.K.) Maza, *P. portoricensis* (Bert.) Small, and *P. segetum* (H.B.K.) Small are chiefly entomophilous but may be of minor importance in allergy.

Chenopodiaceae.—Three genera, *Chenopodium*, *Atriplex*, and *Salicornia*, are allergenic. Mexican tea or wormseed (pazote), *Chenopodium ambrosioides* L., and sowbane, *C. murale* L., are outstanding because they shed large quantities of pollen. Both are widely distributed in Puerto Rico. Various species of this genus grow in Central and South America. Allied species of crested atriplex (garbancillo), *Atriplex pentandra* (Jacq.) Standley, are listed in the literature of the United States (55). They are important causes of pollen allergy, although they do not occur in large enough numbers to replace the major plants. Glasswort (chifle), *Salicornia bigelovii* Torr., assumes a secondary role in localities where it is plentiful (55).

Amaranthaceae.—In Puerto Rico there are three toxic genera: *Amaranthus*,

Celosia, and *Iresine*. The first of these consists of six species: *A. crassipes* Schlecht.; (blero), *A. dubius* Mart.; (blero blanco), *A. viridis* L.; (blero manso), *A. gracilis* Desf.; spiny amaranth (blero espinoso), *A. spinosus* L.; and (gusano), *A. cruentus* L. This genus grows plentifully in the Americas, and all species are of primary consideration when they occur in large numbers. The second genus consists of silvery celosia (albahaca plateada), *C. argentea* L.; (albahaca), *C. nitida* Vahl.; and cockscomb (cresta de gallo), *C. cristata* L. The latter plant is reported as a contributor to the Brazilian pollens (28). In the United States these plants are not sufficiently distributed to include them as essential hay-fever agents, but in Puerto Rico they may be of potential importance. In the third genus, *I. celosia* L. and bloodleaf, *I. paniculata* Kuntze, are probably of minor consequence, although the pollens of bloodleaf are enumerated in a pollen survey of Miami (27).

Plantaginaceae.—Common plantain (llantén), *Plantago major* L., and English plantain or rib grass (yerba de costilla), *P. lanceolata* L., predominate as pollen-producing plants in most of the Americas. They do not grow extensively on the island but might be local contributors to pollen allergy.

Compositae.—The ragweeds (common, great, western, and southern), eminent factors in the pollinosis of the United States, Mexico, Brazil, and Argentina, are lacking in Puerto Rico. However, there are two species of *Ambrosia* on the island. The bay tansy or seashore ragweed, *A. hispida* Pursh., is not widespread and so is only of local importance even though it flowers most of the year. DURHAM (14) reported that it is a contributing factor to the atmospheric pollen in Florida and other Gulf States. (Altamisa, artemisa),

A. peruviana Willd., is of probable importance when it occurs in fairly large numbers, since all species of *Ambrosia* are generally regarded as possessing equal toxicity.

The only species of *Xanthium* growing on the island is cocklebur (bardana), *X. chinense* Mill. The pollens of the various species of this genus interact with one another and all are equally toxic (16). Wormwood (ajenjo), *Artemisia absinthium* L., is of minor interest to the allergists of the United States chiefly because the plants are not abundant. Wormwood, however, is prevalent in some parts of the island and may be a factor in the hay-fever problem. No other representatives of this genus are present.

The other composite plants that have been mentioned in hay-fever literature are chiefly entomophilous and can be considered only as local problems: sunflower (girasol), *Helianthus annuus* L.; globe artichoke (cardo alcachofero), *Cynara scolymus* L.; feverfew *Matricaria parthenium* L.; mugwort (yerba amarga, ajenjo cimarrón), *Parthenium hysterophorus* L.; thistle (cardo), *Carduus mexicanus* Moric.; and fireweed (achicoria de cabra), *Senecio hieracifolia* (L.) Raf.

Typhaceae.—Narrow-leaved cattail (enea), *Typha angustifolia* L., sheds a copious amount of pollen, but in the United States it is of little import in pollen allergy. It is listed, however, among the plants of lesser importance in Argentina (26).

Cyperaceae.—Species related to *Carex polystachya* Sw. are noted in the United States (55), Mexico (44), and Argentina (26). Their abundant pollens are frequently caught on atmospheric slides and are considered allergenic.

Urticaceae.—The artillery plant or gale of wind (madre selva, verdolaguilla), *Pilea microphylla* (L.) Liebm., and

the many species of *Urtica* (Tourn.) L. have been considered as lesser causes of hay fever in the United States (18) and also in Argentina (26).

TREES AND SHRUBS.—Most species of trees and shrubs associated with hay fever in the various parts of the United States (5, 15, 25, 37, 55) are not found in Puerto Rico.

Arecaceae.—The Puerto Rican royal palm (palma real), *Roystonea borinquena* Cook, may be considered allergenic, since the royal palms of Cuba (39) and of Hawaii (40) are causes of allergy. The coconut palm (palma de coco), *Cocos nucifera* L., is a factor in increasing the pollen count of the atmosphere in Miami (12). Pollens of various palms occur on atmospheric slides in Brazil (33). Hence, on the island, the endemic hat palm (palma de sombrero), *Sabal causiarum* (Cook) Beccari, and additional types of palms may be prominent in atmospheric pollen determinations. The allergenic date palms, however, are absent.

Casuarinaceae.—The Australian pine or beefwood tree (pino), *Casuarina equisetifolia* Forst., is reported as a cause of hay fever in Miami (56) and is listed as an allergenic tree of Argentina (26).

Juglandaceae.—Since the walnuts generally are recognized as hay-fever trees, the walnut (nogal, palo de nuez), *Juglans jamaicensis* C., may be of local consequence.

Myricaceae.—The bayberry or candleberry myrtle (cererro, arrayan), *Myrica cerifera* L., is reported as the chief cause of allergy in Bermuda (17) and should be regarded in a similar capacity in Puerto Rico.

Urticaceae.—A hackberry (almez), *Celtis trinervia* Lam., is related to the allergenic species that occur in the United States (55) and in Argentina (9). The tala tree, so prominent in the pollinosis

of South America, does not grow in Puerto Rico.

Moraceae.—Indian laurel (laurel de India), *Ficus nitida* Thunb., has monoecious flowers and may be of consequence in pollen allergy. Fustic (mora, palo de mora), *Chlorophora tinctoria* (L.) Gaud., is insect-pollinated, but its pollens can be carried short distances by the wind. It is related to the paper mulberry, well known to the allergists of North and South America.

Mimosaceae.—The genera *Prosopis*, *Acacia*, and *Albizzia* are allergenic in the United States (12) and in Argentina (26). In addition, *Prosopis* is common in Mexico (44), and *Acacia* in Mexico (44) and in Cuba (39).

Honey mesquite (algaroba), *Prosopis juliflora* (Sw.) DC., is considered to be the most important tree causing hay fever in western Texas (45).

Five species of *Acacia* grow abundantly on the island. None of these occurs in the United States, and none has been reported in hay-fever literature. However, of the various North American species, ROWE (41) stated that *Acacia* pollen is found on atmospheric slides and causes hay fever and asthma. Thus, the wild tamarind (cacia), *A. macracantha* H. & B.; catch-and-keep, *A. riparia* H.B.K.; catechu, *A. suma* Roxb.; spineless acacia (tamarindo cimarrón, acacia nudosa), *A. muricata* (L.) Willd.; and *A. anegadensis* Britton may be of potential danger.

(Aroma, casha), *Vachellia farnesiana* (L.) Wight & Arn., is synonymous with opopanax or popinack, *Acacia farnesiana* Willd. It is entomophilous, but its pollen is also carried by the wind. Severe allergic symptoms were relieved in a hypersensitive child when a number of these trees were cut down near her home.²

² Personal communication.

The yellow acacia (acacia amarilla, amor platónico), *Albizzia lebbek* (L.) Benth., is allied to a mainland species, the mimosa tree, which is suspected of causing hay fever (55). Suspicion is thus cast upon the yellow acacia in Puerto Rico. It is possible that, in addition to the pollens of all these leguminous plants, the odors or "osmyls" described by URBACH (50) are responsible for the production of allergic symptoms.

Meliaceae.—The Indian lilac or pride of India (lilaila, pasilla), *Melia azedarach* L., does not grow in the United States, but it occurs in Argentina. Although it is entomophilous, it can produce allergy (52).

Anacardiaceae.—The pollen of the mango (mangó), *Mangifera indica* L., is mentioned among the wind-borne pollens of Hawaii (40) and of Cuba (39).

Malvaceae.—Among the many species and varieties of hibiscus in Puerto Rico is the Chinese rose (amapola, candelada, pavona), *Hibiscus rosa-sinensis* L. It is insect-pollinated, but that its pollens may cause allergy was indicated by RODDIS (40), who cited one such case in Hawaii.

Terminaliaceae.—Indian almond or malabar almond (almendron), *Terminalia catappa* L., possesses apetalous flowers which may liberate sufficient pollen to be of concern in allergy.

Myrtaceae.—Seven species of the blue

gum (eucalypto), *Eucalyptus robusta* Smith, *E. rostrata* Schl., *E. citriodora* Hook., *E. tereticornis* Smith, *E. viminalis* Labill., *E. resinifera* Smith, and *E. paniculata* Smith grow in Puerto Rico. These are generally considered entomophilous, although *Eucalyptus* pollens have been reported in the atmosphere of Argentina (26) and of Mexico (44).

Compositae.—The broombush, *Baccharis dioica* Vahl., is related to the groundsel bush, which is of importance in Miami (27) and in Mexico (44).

Others.—The following trees and shrubs are chiefly entomophilous, but their pollens can be carried short distances by the wind: West Indian snowberry (bejuco de berac), *Chiococca alba* (L.) Hitchc.; castor-oil plant (higuerito), *Ricinus communis* L.; and three species of *Jasminum*: Arabian jasmine (jasmín oloroso, diamela), *J. sambac* (L.) Soland., hairy jasmine (jasmín de papel), *J. pubescens* (Retz) Willd., and (jasmín de trapo), *J. azoricum* L.

A summary list follows of the species of probable allergenicity in Puerto Rico. The significance of the numerical designations is: (1) of major importance in the pollen allergy of other countries; (2) of minor importance in the pollen allergy of other countries; (3) related to allergenic species of other countries; and (4) contribute pollen to the atmosphere but not proved allergenic.

GRASSES:

- | | | |
|--------------------------------|-----------------------------------|----------------------------------|
| (3) <i>Andropogon bicornis</i> | (3) <i>Chloris ciliata</i> | (3) <i>Paspalum virgatum</i> |
| (3) <i>A. glomeratus</i> | (3) <i>C. inflata</i> | (4) <i>Pennisetum purpureum</i> |
| (3) <i>A. leucostachyus</i> | (3) <i>C. petraera</i> | (4) <i>Phragmites communis</i> |
| (3) <i>A. virgatus</i> | (3) <i>C. radiata</i> | (1) <i>Saccharum officinarum</i> |
| (1) <i>A. virginicus</i> | (1) <i>Cynodon dactylon</i> | (3) <i>Setaria geniculata</i> |
| (4) <i>Arundo donax</i> | (2) <i>Digitaria sanguinalis</i> | (3) <i>S. setosa</i> |
| (2) <i>Avena sativa</i> | (2) <i>Echinochloa crus-galli</i> | (3) <i>Sorghastrum setosum</i> |
| (4) <i>Axonopus aureus</i> | (2) <i>Eleusine indica</i> | (1) <i>Sorghum halepense</i> |
| (4) <i>A. compressus</i> | (4) <i>Eriochloa polystachya</i> | (2) <i>S. vulgare</i> |
| (3) <i>Bouteloua americana</i> | (4) <i>Melinis minutiflora</i> | (3) <i>Sporobolus cubensis</i> |
| (3) <i>B. heterostega</i> | (4) <i>Panicum maximum</i> | (3) <i>S. indicus</i> |
| | (4) <i>P. purpurascens</i> | (1) <i>S. poiretii</i> |

GRASSES—*continued*

- (3) *S. pyramidatus*
- (3) *S. virginicus*
- (2) *Stenotaphrum secundatum*
- (1) *Tricholaene rosca*
- (4) *Tripsacum laxum*
- (3) *Uniola laxa*
- (3) *Vetiveria zizanioides*
- (2) *Zea mays*

HERBACEOUS PLANTS:

- (1) *Amaranthus crassipes*
- (1) *A. cruentus*
- (1) *A. dubius*
- (1) *A. gracilis*
- (1) *A. spinosus*
- (1) *A. viridis*
- (1) *Ambrosia hispida*
- (3) *A. peruviana*
- (2) *Artemisia absinthium*
- (3) *Atriplex pentandra*
- (2) *Carduus mexicanus*
- (3) *Carex polystachya*
- (2) *Celosia argentea*
- (2) *C. cristata*
- (2) *C. nitida*
- (1) *Chenopodium ambrosioides*

- (1) *C. murale*
- (2) *Cynara scolymus*
- (2) *Helianthus annuus*
- (3) *Iresine celosia*
- (2) *I. paniculata*
- (2) *Matricaria parthenium*
- (2) *Parthenium hysterophorus*
- (2) *Persicaria acuminata*
- (3) *P. portoricensis*
- (2) *P. punctata*
- (2) *P. segetum*
- (2) *Pilea microphylla*
- (1) *Plantago lanceolata*
- (1) *P. major*
- (1) *Rumex crispus*
- (2) *Salicornia bigelovii*
- (2) *Senecio hieracifolia*
- (2) *Typha angustifolia*
- (2) *Urtica* spp.
- (3) *Xanthium chinense*

TREES AND SHRUBS:

- (3) *Acacia anegadensis*
- (3) *A. macracantha*
- (3) *A. muricata*
- (3) *A. riparia*
- (3) *A. suma*
- (3) *Albizzia lebbek*

- (3) *Baccharis dioica*
- (1) *Casuarina equisetifolia*
- (3) *Celtis trinervia*
- (4) *Chiococca alba*
- (3) *Chlorophora tinctoria*
- (1) *Cocos nucifera*
- (4) *Eucalyptus citriodora*
- (4) *E. paniculata*
- (4) *E. resinifera*
- (4) *E. robusta*
- (4) *E. rostrata*
- (4) *E. tereticornis*
- (4) *E. viminalis*
- (4) *Ficus nitida*
- (2) *Hibiscus rosa-sinensis*
- (4) *Jasminum azoricum*
- (4) *J. pubescens*
- (4) *J. sambac*
- (3) *Juglans jamaicensis*
- (4) *Mangifera indica*
- (2) *Melia azedarach*
- (1) *Myrica cerifera*
- (1) *Prosopis juliflora*
- (4) *Ricinus communis*
- (3) *Roystonea borinquena*
- (4) *Sabal causiarum*
- (4) *Terminalia catappa*
- (2) *Vachellia farnesiana*

Summary

1. A survey of the flora of Puerto Rico has disclosed the existence of certain anemophilous plants which may be of potential danger to allergic individuals. There are no reports, however, of clinical studies of the pollen allergies caused by these plants.

2. Some of the wind-pollinated species growing in Puerto Rico are identical with those of the United States, Mexico, Argentina, Brazil, Uruguay, Cuba, and Bermuda. It has been determined in the latter locations that many of these plants cause pollinosis. Other island plants are related to the allergenic species of those countries; and still others, although unrelated, contribute a large amount of pol-

len to the atmosphere. Thus, they may prove to be definite factors in pollen allergy.

3. The anemophilous plants are discussed, and comparisons are made. Lists of the grasses, herbaceous plants, and trees and shrubs of probable allergenicity are presented.

4. This investigation suggests as a basis for future clinical study the relationship between the uninvestigated anemophilous plants of Puerto Rico and the allergenic plants of North, Central, and South America.

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A HAPLO-VIABLE DEFICIENCY-DUPLICATION FROM AN INTERCHANGE IN TRITICUM MONOCOCCUM¹

LUTHER SMITH

Introduction

The deficiency-duplication herein reported arises at meiosis from the segregation of chromosomes involved in a heterozygous reciprocal translocation in Einkorn (*Triticum monococcum* L., $n = 7$). The fact that a gametophyte with a deficiency is functional in this diploid

wheat is of interest in itself and suggests that there may be duplicated chromosomes or parts of chromosomes in this species and that the basic number of chromosomes in wheat may be less than seven. The deficiency-duplication is of interest also because of the practical use that can be made of the altered transmission of factors in the deficient and duplicated chromosomes. In nonpolyploid species a deficiency-duplication complex should have an advantage over

¹ Missouri College of Agriculture, journal series paper no. 1018. Investigation carried out at Columbia, Missouri, under auspices of United States Department of Agriculture and Missouri Agricultural Experiment Station.

trisomes in the study of inheritance of factors for disease resistance and other characters difficult to study by usual methods, because one could be selected that had less effect on viability.

Observations

ORIGIN AND DESCRIPTION OF PLANTS WITH DEFICIENCY-DUPLICATION.—A num-

when selfed, produced three types of offspring: (a) Somewhat less than half had seven pairs of chromosomes at meiosis and appeared normal. (b) A corresponding proportion had a ring (or chain) of four chromosomes and were also normal in external appearance. (c) The remainder, or about 7% (based on a population of 900 plants, of which 102 were

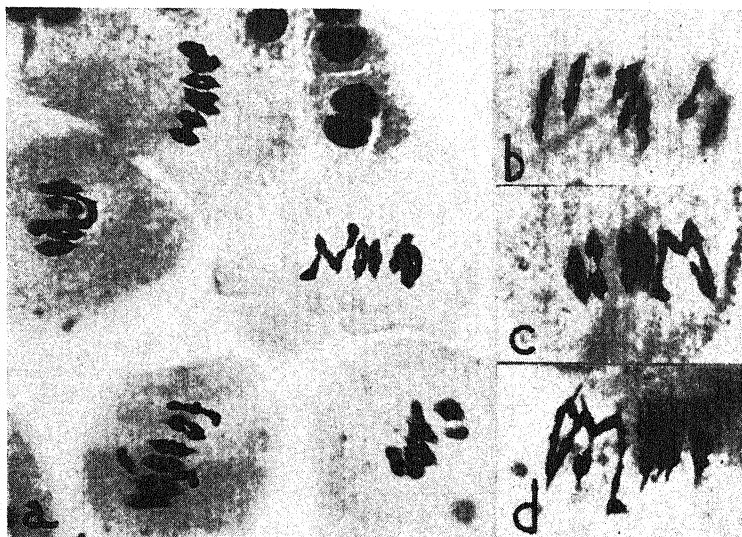


FIG. 1.—Arrangements of chromosomes at first meiotic metaphase in pollen mother cells of plants with deficiency-duplication. (a), group of five cells from anther, showing various configurations of chromosomes; at 3:00 o'clock, zigzag chain of four chromosomes and probably one open and four closed pairs; at 4:00 o'clock, slightly out of focus, one open and six closed pairs; at 6:00 o'clock, two open and five closed pairs; at 9:00 o'clock, open chain of four chromosomes and probably five closed pairs; at 12:00 o'clock, seven closed pairs. $\times 420$. (b), cell from plant with deficiency-duplication showing seven closed pairs. $\times 850$. (c), cell from same plant as b showing attachment between two pairs, presumably as result of pairing among three homologous segments. $\times 850$. (d), configuration similar to that shown in c, but from plant in which three pairs of chromosomes were involved in complex. $\times 850$.

ber of reciprocal translocations were obtained as a result of X-ray treatments of dormant seeds of einkorn (11, 12). Plants heterozygous for one of these, hereafter referred to as "Ring no. 12," had a chain of four chromosomes in 56% and a ring of four chromosomes in 44% of the 275 pollen mother cells tabulated, which suggested that one of the translocated segments was relatively short.

Plants heterozygous for Ring no. 12,

examined cytologically, had seven pairs of chromosomes in some pollen mother cells and five pairs, plus a chain of four chromosomes, in others. In some cells two pairs were attached but not in a manner to be classed as a chain of four (see fig. 1 for types of configurations of chromosomes observed in plants with deficiency-duplication). Consideration of the evidence has led to the conclusion that plants with five pairs plus a chain of

four chromosomes in some pollen mother cells and seven pairs in others had received a gamete with a deficiency and a duplication resulting from the segregation of chromosomes in the heterozygous interchange. "Deficiency-duplication" is a term used to designate such gametes

normal plants seemed identical. In table 1 are presented data on the arrangements of chromosomes at microsporogenesis in normal, Ring no. 12, and deficiency-duplication sib plants.

Sixty-five plants with the deficiency-duplication averaged 79 cm. in height

TABLE 1
ARRANGEMENTS OF CHROMOSOMES IN NORMAL, RING NO. 12,
AND DEFICIENCY-DUPPLICATION SIB PLANTS

PLANTS	NO. OF POLLEN MOTHER CELLS WITH INDICATED ARRANGEMENTS OF CHROMOSOMES						
	7 pairs, closed	7 pairs, 1 open	7 pairs, 2 open	7 pairs, 3 open	Ring of 4	Chain of 4	Unclassi- fiable
Normal.....	200	41	7	0	*	*	
Ring no. 12.....	0	0	0	0	121	154	4
Deficiency-duplication..	33	140	37	4	0	167	122 69

* Not examined critically for rings or chains of more than two chromosomes.

TABLE 2
TRANSMISSION OF DEFICIENCY-DUPPLICATION IN EINKORN

PARENT PLANTS		CONSTITUTION OF PROGENY (NO.)				
♀	♂	Normal	Def.-dup.	Haploid	Triploid	Total
Normal	Normal	697	0	0	0	697 (0)†
Normal	Ring no. 12	986*	2	36‡	2	1026 (101)
Normal	Def.-dup.	92	0	1	0	93 (3)
Ring no. 12	Normal	15*	2	0	0	17 (17)
Ring no. 12	Ring no. 12	824*	59	0	3	886 (102)
Def.-dup.	Normal	240	117	11	13	381 (94)
Def.-dup.	Def.-dup.	460	309	2	22	793 (270)

* "Normals" were "diploid, not deficiency-duplication," but included ring-forming as well as 7-pair plants, approximately half of which were presumably homozygous for the interchanged chromosomes.

† Numbers in parentheses indicate number of plants examined cytologically.

‡ Some but not all these haploids were result of delayed pollination (13).

and the plants that receive them. Plants heterozygous for the deficiency-duplication (no homozygotes were observed) were somewhat slow in development, had fine leaves and spikes, and had small and somewhat defective pollen. Apparently only one of the possible types of gametes receiving deficiency-duplication complexes was viable, because the meiotic and external appearance of all the ab-

and bore eighteen culms each as compared with 86 cm. and sixteen culms for each of 151 normal sibs. They resembled haploids except that they were larger, more vigorous, and fairly fertile. The fertility of four exceptionally vigorous plants with the deficiency-duplication (including both primary and secondary florets) was 49% as compared with 54% for four normal, and with 62% for one

ring-forming sib. The numbers of florets tabulated were 632, 893, and 340, respectively.

TRANSMISSION OF DEFICIENCY-DUPLICATION COMPLEX.—Data from tests of sib plants dealing with the inheritance of the deficiency-duplication complex are presented in table 2. Of the nine possible combinations of the three types of female and male parents, all were tested except the reciprocal cross between plants with the deficiency-duplication and Ring no. 12. These two combinations would give little information on transmission of the complex. The other tests showed that transmission of the deficiency-duplication was almost entirely through the female gametes of plants with Ring no. 12 (7%) or with the deficiency-duplication (30-40%).

There were two instances (among 1119 plants in which it could be detected) of transmission of the deficiency-duplication through the pollen of Ring no. 12 plants. These two individuals had the typical external appearance and meiotic behavior of plants with the complex.

Plants with the deficiency-duplication produced an unusual proportion of haploid and triploid progeny. Most, and perhaps all, of the triploids had the deficiency-duplication. This was indicated by macroscopic and microscopic observations, but it was not determined whether they had one or two chromosomes with the deficiency-duplication.

EFFECT OF DEFICIENCY-DUPLICATION COMPLEX ON INHERITANCE OF FACTORS FOR MUTANT CHARACTERS.—The presumed derivation and chromosomal constitution of plants with the deficiency-duplication are shown in figure 2. The more common constitution, obtained by crossing a plant having the deficiency-duplication with a normal (also present

in half the deficiency-duplication plants in selfed progenies of plants heterozygous for Ring no. 12), is shown in *d* and *e*. Hypothetical factors are shown in *d* and

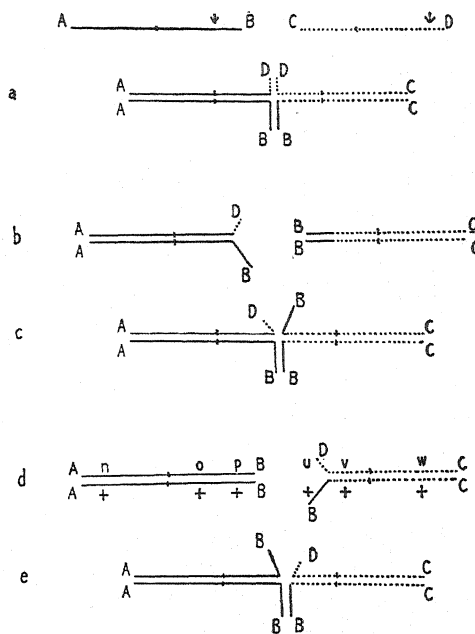


FIG. 2.—Diagram showing derivation and chromosomal constitution of plants with some pollen mother cells having seven pairs of chromosomes and others having five pairs plus chain of four. (a), association of four chromosomes resulting from interchange between two nonhomologous chromosomes at points indicated by arrows. Points of interchange are hypothetical. One type of separation would produce viable spores with chromosomes A B C B, which would be deficient for only small segment D and have a duplication for segment B, (b and c), configurations of chromosomes in pollen mother cells of plants receiving A B C B and interchanged chromosomes A D C B. There would be either (b) seven pairs of chromosomes (here and elsewhere in this paper unaffected chromosomes are referred to but not diagrammed) with one pair usually open, D A A B, or (c) five pairs plus chain of four, D A A B B C C B. (d and e), configurations of chromosomes in pollen mother cells of plants receiving A B C B and normal chromosomes A B C D. There would be (d) seven pairs of chromosomes with one pair usually open, D C C B, or (e) five pairs plus a chain of four, B A A B B C C D.

may be used to illustrate the effect of the deficiency-duplication on the inheritance of factors at various loci along the chromosomes.

A recessive factor (for example, *u*) in the region of the normal chromosome, corresponding to the segment missing from the deficient chromosome, should appear as a "pseudo-dominant" in plants with the deficiency-duplication. A factor (for example, *v*) in the same general region of the chromosome, whether or not opposite the deficient segment, would segregate in higher than normal ratio be-

cause of the competitive advantage of gametophytes with normal chromosomes over gametophytes with the deficiency-duplication.

A factor (for example, *w*) far enough removed from the deficiency-duplication to approach 50% crossing over with it would segregate in normal ratio. Characters determined by factors in the triplicated segment would segregate in approximately 7:1, 3:1, and 1:1 (not trisomic) ratios in selfed and in the two kinds of reciprocally backcrossed progenies because of the difference in functioning of male and

TABLE 3
EFFECT OF DEFICIENCY-DUPPLICATION ON INHERITANCE OF MUTANT CHARACTERS

LINKAGE GROUP*	PARENT PLANTS†	FACTOR SEGRE-GATING	CONSTITUTION OF PROGENY				
			Total (no.)	Dominant phenotype (no.)	Recessive phenotype		
					No.	%	Standard deviation
A.....	Normal	<i>j</i>	2839	2082	757	27	1
A.....	Def.-dup.	<i>j</i>	231	178	53	23	3
A.....	Normal	<i>gl-2</i>	3390	2652	738	22	1
A.....	Def.-dup.	<i>gl-2</i>	55	46	9	16	5
B.....	Normal	<i>gl-1</i>	2121	1612	509	24	1
B.....	Def.-dup.	<i>gl-1</i>	275	214	61	22	3
B.....	Normal	<i>wi</i>	2141	1647	494	23	1
B.....	Def.-dup.	<i>wi</i>	242	177	65	27	3
C.....	Normal	<i>yg</i>	2510	1949	561	22	1
C.....	Def.-dup.	<i>yg</i>	183	151	32	17	3
C.....	Normal	<i>e-2</i>	3683	2927	756	21	1
C.....	Def.-dup.	<i>e-2</i>	117	87	30	26	4
C.....	Normal	<i>yx-2</i>	1413	1108	305	22	1
C.....	Def.-dup.	<i>yx-2</i>	147	117	30	20	4
D.....	Normal	<i>y</i>	3090	2309	780	25	1
D.....	Def.-dup.	<i>y</i>	322	260	62	19	2
D.....	Normal	<i>ga</i>	959	750	209	22	1
D.....	Def.-dup.	<i>ga</i>	199	155	44	22	3
D.....	Normal	<i>js</i>	1478	1164	314	21	1
D.....	Def.-dup.	<i>js</i>	16	13	3	19	10
D.....	Normal	<i>cx-1</i>	804	643	161	20	1
D.....	Def.-dup.	<i>cx-1</i>	160	129	31	19	3
F.....	Normal	<i>g</i>	1434	1064	370	26	1
F.....	Def.-dup.	<i>g</i>	199	156	43	22	3
	Normal	<i>e-1</i>	5227	4447	780	15	1
	Def.-dup.	<i>e-1</i>	402	262	140	35	2
	Normal	<i>ar-2</i>	1157	963	194	17	1
	Def.-dup.	<i>ar-2</i>	601	370	231	38	3

* See fig. 3 for linkage group.

† Normals were not necessarily sibs of deficiency-duplication plants.

female germ cells with the deficiency-duplication. Factors (for example, *o* and *n*) not included in the triplicated segment in the homologous bivalent should segregate in normal ratios.

Fourteen factors (table 3 and fig. 3) were tested for "pseudo-dominance" and

Normal plants heterozygous for *e-1* or *ar-2* (as well as plants heterozygous for certain other factors in other chromosomes) had significantly less than 25% of the progeny with the recessive phenotype. This was not due to poor classifiability, at least in the case of *e-1 e-1*

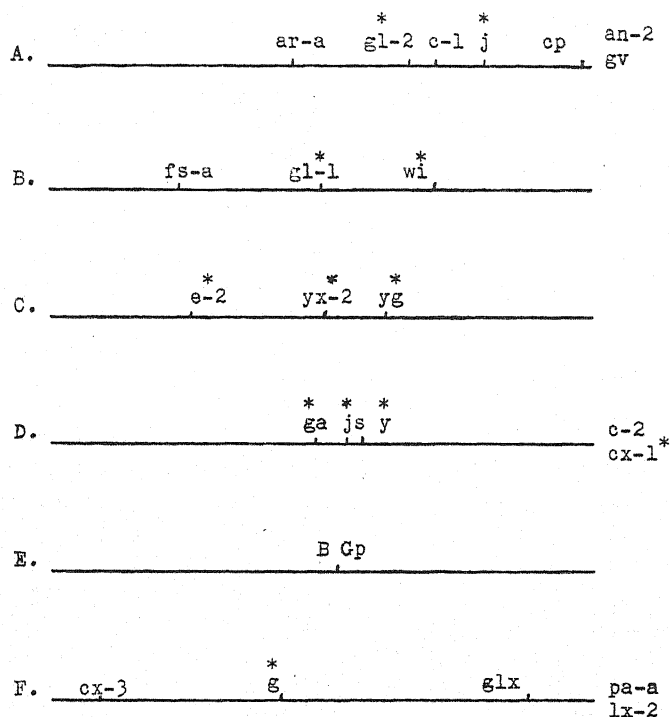


FIG. 3.—Linkage map of factors known to be linked in einkorn (see SMITH [11] for descriptions of mutant characters). One inch represents approximately 15 crossover units. Orders of some of factors on chart (as well as all factors on right-hand margin) were not definitely established. Groups of linked factors are not necessarily on different chromosomes. However, one or more factors in each group (and *e-1*) were tested with factors in each of the other groups and found to be apparently unlinked. An asterisk (*) indicates factors tested for "pseudo-dominance" and abnormal segregation in plants with deficiency-duplication.

for abnormal segregation. None showed "pseudo-dominance" but two (*e-1* and *ar-2*) segregated in unusual ratios in the selfed progeny of plants with the deficiency-duplication. The results indicated that *e-1* and *ar-2* are in the same pair of chromosomes (CD in fig. 2), but tests on the linkage relations of these factors in normal plants have not been completed.

plants, and was probably not due to poor viability. Apparently a considerable number of genes, like the *wx* gene in maize (14), affect the transmission of the chromosomes containing them.

Discussion

The fact that a gamete with the deficiency-duplication is viable in einkorn suggests that the species may have dup-

lications of segments or whole chromosomes. Such a conclusion would be difficult to substantiate with evidence. The basic number of chromosomes in einkorn has already been discussed by SMITH (13), but it may be added here that even if it were proved that pairing in haploids of this species occurs between two particular chromosomes (or parts of chromosomes), or that any additional viable deficiency-duplication complexes involved these same chromosomes, there would still be no indubitable proof that the basic number of chromosomes is less than seven.

Whatever the explanation of the deficiency-duplication may be, the results provide a very useful application of the inheritance of factors in the affected chromosomes. The deficiency-duplication complex should prove of use in locating factors in ordinary linkage mapping and of value in studying the factorial foundation of such quantitative characters as disease resistance, quality, and yield. The deficiency-duplication would so alter the inheritance of such factors (in species where the method is practicable) that it should be possible to study their inheritance effectively.

In most diploid species in which a large deficiency would probably not be tolerated in the gametophyte generation, the interchanges most likely to provide the viable deficiency-duplication segregates desired would be those appearing as a chain, instead of a ring, of four chromosomes at meiosis in a considerable proportion of the pollen mother cells. To be most useful, the plants with the deficiency-duplication should be distinguishable macroscopically, in order to obviate the necessity of identifying the affected plants cytologically.

If the deficiency-duplication complex could not be associated with other chro-

mosomes, its usefulness would be limited, because additional, viable deficiency-duplication complexes would have to be found for the various chromosomes. It is possible, however, by inducing another interchange, to associate the deficiency-duplication with any third pair of chromosomes to form a chain of six (chain of four in some sporocytes). Thus, a single viable deficiency-duplication would suffice in a species for altering the transmission of any chromosome of the genome.

There are four possible types of interchange involving a different arm of the two pairs of chromosomes in the deficiency-duplication complex with a third pair of chromosomes, but only the most useful type is illustrated in figure 4.² A plant with the arrangement of chromosomes shown would be approximately semisterile (if segregation were not directed) as compared with (potentially) full fertility in a deficiency-duplication plant with seven pairs or a chain of four plus five pairs. However, the only viable gametes (exclusive of certain kinds of crossovers) would be those with a normal set of chromosomes and those with the interchanges and deficiency-duplication. Thus, any genetic factors linked with the deficiency-duplication in the chromosomes B F and C E would be altered in their transmission. The degree of the change in transmission would depend on the amount of crossing-over between the factors and the interchange or deficiency breaks.

² In addition to interchanges involving both arms of a chromosome, there would, of course, be instances in which the second interchange involved the same arm as the first. To illustrate and discuss all these possibilities, and the effect crossing-over would have on the transmission of factors located at various points on the affected chromosomes, would require a great deal of space. It is felt that the information already given and fig. 4 will suggest to the reader the possibilities of using a deficiency-duplication (or other abnormalities with similar effects on the transmission of chromosomes) in genetic studies.

Crossovers would interfere somewhat with the use of the complex, and it may be necessary to use interchanges with breaks closer to the deficiency-duplication than the one illustrated in figure 4, in order to have transmission of genes in

would be a new chromosome F D, which, if combined with chromosomes of the proper constitution, would result in an entirely new interchange heterozygote.

In this connection it seems desirable to point out that additional observations

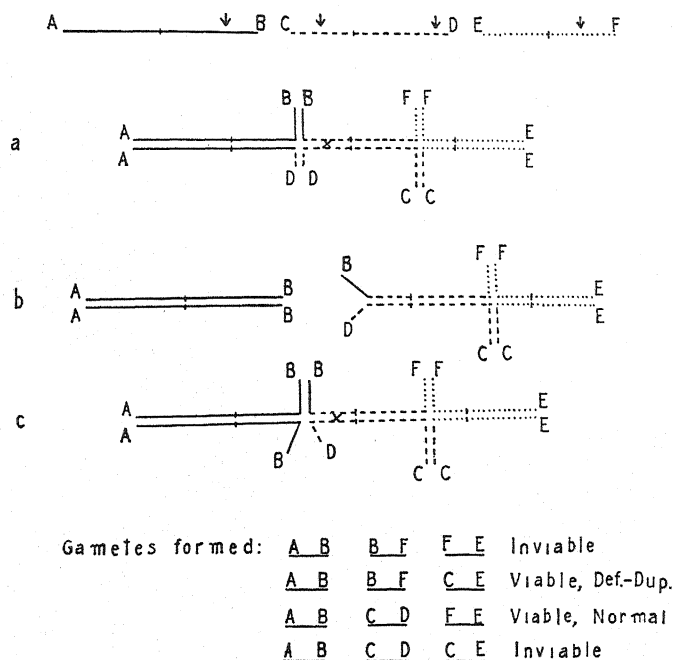


FIG. 4. Diagram showing derivation and chromosomal constitution of plants with deficiency-duplication involving three pairs of chromosomes. (a), association of six chromosomes resulting from interchanges among three nonhomologous chromosomes at points indicated by arrows. One type of separation would produce viable spores with chromosomes A B B F C E which would have same deficiency-duplication as observed in plants with seven pairs or chain of four plus five pairs (fig. 2). (b and c), configurations of chromosomes in pollen mother cells of plants receiving A B B F C E and normal chromosomes A B C D E F. Some cells would have five pairs plus chain of four (b); others four pairs plus chain of six (c). Note that only viable spores (non-crossover) formed by plant with deficiency-duplication would contain normal set of chromosomes or deficiency-duplication complex which would form a chain of six (or four) at meiosis, when combined with normal chromosomes. See text.

both deficiency-duplication and the interchanged chromosomes affected. Also, a crossover at X in either a or c (fig. 4) could result in the production of a plant having a deficiency-duplication involving two instead of three pairs of chromosomes (which, however, should be distinguishable on the basis of fertility). The complementary crossover strand

are needed for an interpretation of the effect of crossing-over on ring and chain configurations at meiotic metaphase in plants heterozygous for interchanges. The current view is that, if an interchange frequently results in a chain instead of in a ring of four, one of the pieces translocated is short. If the piece translocated is short, however, the segment

between the centromere and the point of interchange should, in some chromosomes, be long. In such a long segment the chiasma frequency should be practically unaltered, and there would be chiasmata on both sides of the centromeres of the four chromosomes involved in the interchange (fig. 2). Such a configuration ought to be distinctive (because, supposedly, chiasmata cannot terminalize across centromeres or points of interchange) and worthy of note. In the present study, for example, these configurations, with chiasmata on both sides of the centromeres, should have occurred about as frequently in plants with Ring no. 12 as seven closed pairs occurred in plants with the deficiency-duplication (table 1). This would be expected unless the formation of a ring or chain interfered with (precluded) chiasma formation between the centromeres and the breaks, which seems unlikely. RHOADES (8) also reported observations that did not fit prevailing concepts "relating crossing over to chiasmata and chiasmata to post-diplotene association." Another consequence of crossing-over in heterozygous interchanges is discussed in the next paragraph.

A crossover between the centromere and the break in either or both arms of chromosomes involved in an interchange (e.g., involving two pairs of chromosomes) would be expected to result in a zigzag arrangement of chromosomes at first meiotic metaphase owing to the terminalization of the chiasma. In favorable cells it should be possible to observe the exchange of strands at the point of overlap in the figure-of-eight. Therefore, the proportions of adjacent and alternate disjunctions might vary with different interchanges, depending upon the frequencies of crossing-over between the centromeres and the breaks. Half the

spores from sporocytes in which such a crossover had occurred would have a deficiency and a duplication and (ordinarily) would be nonviable. Thus, in maize, if a zigzag arrangement of the chromosomes at first meiotic metaphase was due to crossing-over between the centromere and the point of interchange, even the zigzag segregates would result in 50% sterility, just as in cases in which adjacent chromosomes went to the same pole.

Evidently, zigzag configurations are not all due to crossing-over (with resulting nonviable spores) in diploid and tetraploid wheat, because the percentage of sterility in plants with a ring of four is considerably less than half the percentage of zigzag configurations.

The frequency of individuals with the deficiency-duplication among the progeny of plants with Ring no. 12 (7%) indicates that the frequency of zigzag configurations of the chromosomes in the ring (or chain) was about 93% (possibly somewhat less, since the deficiency-duplication apparently reduced viability somewhat and since the chromosomes occasionally may have been oriented on the spindle in a different way). This degree of directed segregation approaches the percentage of fertility (89%) observed in plants with a ring (or chain) of four chromosomes (12).

BURNHAM (2) reported a similar case of viable deficiency-duplication in maize arising from an interchange involving only a few chromomeres of the satellited chromosome. The deficiency-duplication behaved in a manner similar to that of the deficiency-duplication reported herein and could presumably be used in the same manner. However, the translocations of the B chromosome studied by ROMAN (9) may prove to be even more useful in determining linkage relations of

genes in maize, since crossing is so easily accomplished in this species. ANDERSON (1) has also pointed out the usefulness of interchanges in maize for the study of linkage relations, especially of factors for traits of economic importance.

SEARS (10) reported a viable deficiency-duplication segregate from a ring of four chromosomes in the hexaploid wheat, *Triticum vulgare*. Plants with a deficiency-duplication should be fairly common in polyploid species of wheat, oats, tobacco, and others in which deficiencies of whole chromosomes or even pairs of chromosomes have been reported. In such polyploids, trisomes, monosomes, or nullisomes could probably be used more efficiently than a deficiency-duplication complex in studies of linkage relations. However, duplicate and triplicate factors, as well as modifiers, will still complicate linkage studies in polyploids.

Few cases of viable deficiencies in diploid species of plants have been recorded. The writer has observed a few instances of viable deficiencies resulting from X-ray treatments of mature pollen and dormant seeds of einkorn (unpublished observations). Haplo-viable deficiencies in maize have also been reported by STADLER (15) to result from X-ray treatments, and CREIGHTON (3) and MCCLINTOCK (4, 5, 6) reported homozygous deficiencies in the same species. Simple deficiencies, such as those reported by STADLER and by others, could be used, in the manner herein described, to determine whether factors were located in the chromosome pair in which the deficiency was located and could be associated with additional chromosomes by means of interchanges. Similar use could be made of fragments of chromosomes, such as the telocentric chromosome in maize studied by RHOADES (7), that could be connected

with any pair of chromosomes by an interchange. The usefulness of these aberrations in locating genes will depend on the extent of the regions of the chromosomes in which the transmission of the genes is altered. This extent will in turn depend upon the amount of crossing-over between the genes and the breaks, which can be determined only by experiment.

Summary

1. In the selfed progeny of plants of *Triticum monococcum*, heterozygous for a reciprocal translocation, about 93% of the plants appeared normal and about 7% abnormal. The abnormal plants were somewhat smaller than normal sibs and had slightly defective pollen and some sterility. Approximately half the normal-appearing plants had seven pairs of chromosomes at meiosis, and the other half were heterozygous for the interchange. Two types of configurations were observed at microsporogenesis in the abnormal-appearing plants. In some pollen mother cells there were five pairs plus a chain of four chromosomes at first metaphase; in other cells there were seven pairs.

2. The evidence supports the assumption that the abnormal plants had received a gamete with a deficiency-duplication resulting from the segregation of the chromosomes in the heterozygous interchange. The deficiency-duplication was transmitted by 30-40% of the female gametes, but by very few of the male gametes. Twelve factors in five linkage groups were not influenced in their inheritance by the deficiency-duplication. Two other factors were found to be greatly influenced in their transmission by the deficiency-duplication. Thus, the complex helped to establish an apparently new linkage group.

3. The deficiency-duplication is of interest (a) because of its occurrence in a nonpolyploid species of wheat and (b) because of the possibility that such an irregularity may be of value in determining the inheritance and linkage relations of genes. The problem of associating the deficiency-duplication with other chro-

mosomes is discussed, and a diagram is presented showing how any third pair of chromosomes could be successfully linked with the complex by means of an interchange.

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MINERAL COMPOSITION OF BEAN STEMS TREATED WITH 3-INDOLEACETIC ACID

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Introduction

Application of 3-indoleacetic acid to stems of bean plants is followed by the movement of sugars and soluble nitrog-

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enous compounds from other parts of the plant to the treated region. Starch is hydrolyzed in the treated region, and correspondingly there is an increase in water-soluble carbohydrates (1, 5, 6, 7, 8, 11).

This paper reports a study of changes in the amounts of nine inorganic elements in stems of bean plants following surface treatment with 3-indoleacetic acid. Zones

about 2 mm. wide located at the mid-points of the first internodes of young bean plants were coated with lanolin paste alone or with lanolin paste containing 2% 3-indoleacetic acid. Samples of the first internodes were taken at intervals after application and spectrochemically analyzed. The objects of the investigation were (a) to determine when statistically significant differences occurred between the amounts of the inorganic elements in nontreated and treated stem sections and (b) to study the rates of accumulation of the different elements in the treated sections.

Material and methods

The "Cal-approved" strain of Red Kidney bean (*Phaseolus vulgaris*) was grown in the greenhouse in clean quartz sand in 1-quart glazed crocks, each containing two plants. A nutrient solution with essential minor elements was supplied at suitable intervals. When the first internodes were about 16 mm. long, the plants were selected for uniformity of height and were divided into two groups. In the first group, lanolin alone was applied in a zone approximately 2 mm. wide around the first internode, midway between the first and second nodes. The second group of plants was similarly treated with a lanolin paste containing 2% 3-indoleacetic acid. The treated and nontreated (control) plants were randomized on a greenhouse bench. Samples of first internodes were taken for analysis 0, 12, 30, 48, 72, 100, and 148 hours following application. Up to and including the 30-hour sampling, forty plants each were taken for analysis from control and treated lots; thereafter twenty plants were taken each time from each group. Sampling was started at 9:00 A.M. on March 27. The daily light intensities up

to and including April 2 were: 452, 345, 368, 545, 427, 115, and 509 gram-calories/cm².

At each sampling the lengths of first internodes and total plant heights were measured. Adherent lanolin was removed from control and treated stems by wiping the first internodes with a clean cloth. Then sections 1 cm. in length and including the treated regions were cut out of these internodes by means of two razor blades fastened 1 cm. apart in a wooden handle. After measurement of fresh and dry weights of the sections, each one was spectrochemically analyzed.

Each dried stem section was placed in a carbon electrode suitably drilled at one end. The sections taken 48 hours or more following application had increased in dry weight to such an extent, however, that they had to be broken into two to four pieces which were weighed and handled as separate subsamples. The samples placed in the carbon electrodes were ashed at a temperature of 450° C. Each ash residue was evaporated in an electric arc, and the resultant line spectrum was recorded photographically. In addition, each plate recorded spectra in triplicate for 0.1-ml. aliquots from suitable dilutions of a standard solution. The concentrations, expressed as micrograms per 0.1 ml., of the nine elements present were: Ca, 157.8; Mg, 86.3; P, 251.6; K, 977.4; Fe, 13.63; Mn, 4.30; Cu, 1.42; Al, 12.0; and B, 0.736. The transmissions of lines for appropriate wave lengths for the different elements recorded on each plate were measured photoelectrically. From the calculated densities of standard lines, density-log concentration curves for each element were plotted. From knowledge of the line densities of these elements in samples spectra, the corresponding amount of a given element could then readily be determined. Additional details of the

spectrochemical procedure have been described (2, 3).

Results

GROWTH DATA

Highly significant increases⁶ in lengths of treated first internodes occurred except in samples taken 100 hours after application (table 1). Highly significant successive increases in total plant heights occurred only in samples taken 12, 30, and 72 hours following application. These

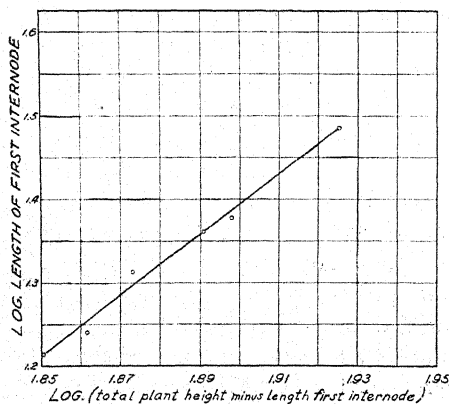


FIG. 1.—In control plants, relation between length (mm.) of first internode and length of remainder of plant follows a power law for first 100 hours. This growth regularity was absent in treated plants (see table 1).

differences apparently were not correlated with light intensities over the experimental period.

In the control plants the lengths of first internodes were related to total plant heights minus lengths of first internodes in a way that can be closely described for the first 100 hours after application by equation (I) (fig. 1):

$$y = 0.00000332 X^{3.65} \quad (I)$$

The maximum deviation observed from calculated values was less than 5%.

⁶ A significant difference in all cases means statistical significance at the 5% level. Highly significant differences have statistical significance at the 1% level or lower.

The equation indicates that, during the period considered, the first internode of an untreated plant elongated more rapidly than the rest of the plant. A full discussion of the growth of one part of an organism as related to the growth of other parts or to the whole organism has been given by HUXLEY (4) and by NEEDHAM (9). The significant fact in this experiment is that there was a regular growth relation in control plants which was entirely lacking in the treated ones. Table 1 shows that during the interval from 30 to 48 hours treated plants did not increase either in length of first internode or in total height.

Twelve hours after application and subsequently the treated stem sections showed highly significant increases in fresh weight as compared with control sections. In both groups the amount of water increased as a linear function of time, but the slope of the curve for treated sections was 7.2 times as great as the slope of the curve for untreated sections.

$$\begin{aligned} \text{Treated sections: } y &= 38.4 + 1.047t \\ S_y &= 1.29, \quad (II) \end{aligned}$$

$$\begin{aligned} \text{Untreated sections: } y &= 41.7 + 0.146t \\ S_y &= 2.34, \quad (III) \end{aligned}$$

where y is the average weight (mg.) of water in a section, t is the time (hours) following treatment, and S_y is the standard error of estimate of y .

The average height of untreated first internodes was also a linear function of time with a rate of increase of approximately 0.14 mm./hr. The increase in height paralleled the increase in water. In marked contrast, dry weights of treated sections increased exponentially with time (fig. 2). The empirical equation involved is

$$W_t = W_0 e^{0.01t} \quad (IV)$$

where W_0 is the average dry weight—4.95 mg.—before treatment and W_t is the average dry weight (mg.) after t hours of treatment. Observed weights deviated on the average from calculated values by 4.9%, with a maximum deviation of 8.2%.

According to equation (IV), the dry weight increased 1%/hr., doubling after 69 hours and quadrupling after 138 hours following application. The equation shows, moreover, that the per-hour rate of increase in dry weight was also exponential with respect to time and was equal to 1% of the dry weight present at any given time.

Table 1 and figure 2 show that changes in dry weights of untreated sections were slight and irregular.

ANALYTICAL DATA

The results of analyses for nine elements (table 2) are stated as micrograms per centimeter of stem length. The data are expressed on a unit-length basis, since questions involving rates of accumulation of absolute amounts of different elements in the stem sections were of primary interest. A percentage basis would be confusing, since it would introduce an additional factor, dry weight, which—as figure 2 clearly shows—increased exponentially with time in treated sections and showed little or no change in controls.

MAGNESIUM.—Thirty hours after application of 3-indoleacetic acid, there was a highly significant increase in the magnesium content of treated stem sections as compared with control sections. The differences in subsequent samplings also were highly significant. Magnesium increased in the treated sections at a constant rate (fig. 3):

$$\text{Mg} = 3.5 + 0.29t \quad S_{\text{Mg}} = 1.92 \quad (\text{V})$$

POTASSIUM.—After 30 hours of treatment, the potassium content of treated sections was greater than in controls. As with magnesium, the difference was highly significant and continued so throughout the experiment. Potassium also increased at a constant rate (fig. 3):

$$\text{K} = 116 + 7.12t \quad S_{\text{K}} = 61.0 \quad (\text{VI})$$

The observed value for 72 hours was only 68% of the calculated value. Since the

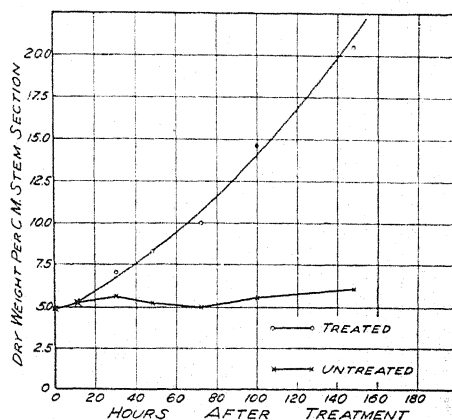


FIG. 2.—Effect of 3-indoleacetic acid on dry weight (mg.) of midsections of first internodes of young bean plants. Dry weight of treated sections increased exponentially with time: $W_t = W_0 e^{0.01t}$.

potassium content for untreated sections at this time was also low, some significant but temporary fluctuation in the environment independent of the hormone treatment was apparently responsible.

PHOSPHORUS.—The treated internodes contained more phosphorus 30 hours after application than did the control sections. In one set of samples this difference was significant, but in a second set the increase lacked significance. All samples taken after 48 hours or more of treatment showed highly significant increases. Phosphorus in treated stem sections increased exponentially with time

TABLE 1
EFFECTS OF TREATING STEMS (MIDDLE OF FIRST INTERNODE) OF BEAN PLANTS
WITH 2% 3-INDOLEACETIC ACID IN LANOLIN PASTE

TIME AFTER APPLICATION (HR.)	LENGTH OF FIRST INTERNODE (MM.)			TOTAL HEIGHT (MM.)			WET WEIGHT (MG.) OF SECTION 1 CM. IN LENGTH			DRY WEIGHT (MG.) OF SECTION 1 CM. IN LENGTH		
	Control	Treated	Diff.	Control	Treated	Diff.	Control	Treated	Diff.	Control	Treated	Diff.
0.....	16.4	87.3	45	4.9
12.....	17.2	20.3	3*	89.7	94.6	5†	48.0	54.1	6.1†	5.2	5.2	0.0
30.....	20.6	27.1	6†	95.1	102.7	8†	52.9	70.9	24.0†	5.6	7.0	1.4†
48.....	23.0	26.0	4†	100.8	102.0	1	55.9	97.2	41.3†	5.2	8.3	3.1†
72.....	23.9	30.6	7†	103.0	113.7	11†	57.4	123.6	66.2†	5.0	10.0	5.0†
100.....	30.6	31.8	2	114.9	114.7	0	61.9	102.4	100.5†	5.6	14.7	9.1†
148.....	30.2	34.4	5†	120.1	122.3	2	68.6	211.8	143.2†	6.1	20.5	14.4†

* Significant at 1.0% level.

† Significant at 0.1% level.

TABLE 2
RESULTS OF SPECTROCHEMICAL ANALYSIS OF KIDNEY BEAN STEMS TREATED WITH
2% 3-INDOLEACETIC ACID IN LANOLIN, WITH CONTROLS

TIME IN HOURS AFTER APPLICATION	CALCIUM		MAGNESIUM		PHOSPHORUS	
	Control	Treated	Control	Treated	Control	Treated
0.....	7.9	5.6	21.9
12.....	11.4	10.2	6.1	6.8	22.6	23.8
30.....	13.8	15.4	6.4	12.1	25.8	29.6
48.....	22.1	23.0	6.9	16.4	22.1	37.9
72.....	25.3	27.1	8.7	24.0	19.5	62.4
100.....	40.6	45.6	7.6	29.3	21.0	76.5
148.....	114.5	103.0	19.0	49.0	16.4	88.0

	POTASSIUM		IRON		COPPER	
	Control	Treated	Control	Treated	Control	Treated
0.....	163	0.390	0.091
12.....	195	204	.333	0.300	.087	0.084
30.....	232	350	.383	0.377	.075	.083
48.....	221	444	.470	0.600	.075	.110
72.....	156	495	.450	0.700	.110	.180
100.....	277	866	.310	0.850	.094	.282
148.....	358	1210	0.400	1.430	0.104	0.232

	MANGANESE		ALUMINUM		BORON	
	Control	Treated	Control	Treated	Control	Treated
0.....	0.17	0.26	0.0140
12.....	.18	0.19	0.33	0.35	.0125	0.0115
30.....	.20	0.26	0.34	0.32	.0112	.0130
48.....	.30	0.59	0.30	0.40	.0102	.0195
72.....	.40	0.93	0.25	0.48	.0087	.0227
100.....	.25	0.88	0.31	0.63	.0054	.0166
148.....	0.72	1.34	2.11	1.97	0.0047	0.0350

for the first 100 hours of treatment (fig. 3).

$$P = 20.81 e^{0.0135t} \quad (\text{VII})$$

The average deviation of the calculated values was 5.7% of the observed ones. A maximum deviation of 11% occurred in the 72-hour sample.

MANGANESE.—A significant increase in the manganese content of treated stem sections was noted 30 hours following application of 3-indoleacetic acid. All subsequent differences were highly significant. The data strongly suggest that manganese accumulated in the treated sections as a linear function of time. Relatively poor precision in the spectrochemical determination of this element prevented critical evaluation of this relation.

COPPER.—Increases in copper content of treated compared with untreated stem sections were first significant 48 hours after application. Subsequent increases were all significant except for the 100-hour samples, in which there was an unusual variability in the analyses.

IRON.—Seventy-two, 100, and 148 hours after application highly significant increases in iron occurred in treated sections in comparison with controls.

ALUMINUM.—Samples taken from treated sections after 72 and 100 hours were significantly greater in their aluminum content than control sections. The marked increases in both control and treated sections in the interval from 100 to 148 hours are noteworthy.

BORON.—At the 30-hour sampling and thereafter, significant increases occurred in the boron content of treated as compared with control sections. The data show that the absolute value of the boron present in the control sections decreased regularly with time, which possibly indicates that it was translocated to the growing points. On the other hand,

the boron content of treated sections increased with time. It should be pointed out that the values for boron recorded in table 2 were obtained by extrapolation of the standard curves, since the boron content in the samples was less than that of the highest dilution of the stock solu-

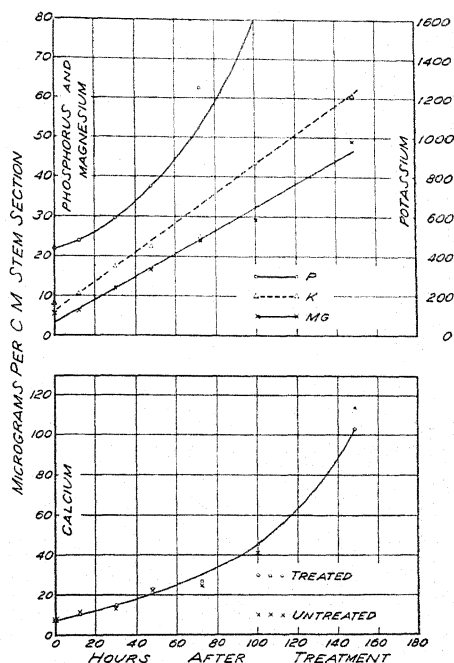


FIG. 3.—Above, effect of 3-indoleacetic acid on amounts of P, Mg, and K in midsections of first internodes of young bean plants (for relatively slight changes in untreated plants see table 2). Below, effect on calcium content. Abscissae are same in both graphs.

tion. Recording the data to the fourth decimal place was considered justifiable in view of the differences so revealed.

CALCIUM.—In contrast to the other elements measured, calcium did not, with one exception, show any significant increases in content as a result of treating stems with 3-indoleacetic acid. One of two subsamples for the 72-hour sampling showed unusually small variability and hence a highly significant difference. Calcium accumulated in both treated

and control sections as an exponential function of time (fig. 3). The equations are:

$$\begin{aligned}\text{Treated sections: } Ca &= 8.66 e^{0.0166t} \\ S_{Ca} &= 3.97, \text{ (VIII)}\end{aligned}$$

$$\begin{aligned}\text{Control sections: } Ca &= 7.80 e^{0.178t} \\ S_{Ca} &= 3.97. \text{ (IX)}\end{aligned}$$

Discussion

It is obvious that the increased growth stimulated by the application of 3-indoleacetic acid required the participation of numerous enzyme systems. A striking phase in the recent development of biochemistry has been the recognition that various metals are essential to many enzymes either as components or as activators. Furthermore, in some cases a metal may strongly inhibit an enzyme (12). Thus carboxylase is a diphosphothiamin-Mg-protein compound, enolase is a Mg-protein compound, polyphenol oxidase is a Cu-protein compound, while iron enters into the structure of catalase, peroxidase, the cytochromes, cytochrome oxidase, and cytochrome peroxidase. Magnesium, calcium, and manganese activate a number of enzyme systems. Catalysed additions of the phosphate radical to, and subtraction from, various molecules are attended by important energy changes.

One would then expect that growth of bean stems stimulated by the application of 3-indoleacetic acid would involve the mobilization and accumulation of various metals, quite apart from the participation of the elements in protoplasmic structure (e.g., phosphorus in phospholipids and nucleoproteins).

Highly significant increases in potassium, magnesium, manganese, and boron were detectable 30 hours following application of 3-indoleacetic acid. By this time, the initial dry weight of treated

stem sections had increased 43%, as contrasted to 14% for control sections. Prior to the very significant increase in dry weight demonstrated after 30 hours, there was a highly significant increase in fresh weight within 12 hours after application.

In comparison with other elements in the treated sections, mobilization and accumulation of copper and iron were delayed. It is probable that the use of more precise analytical methods and greater care in guarding against contamination of the samples by extraneous iron would have resulted in the demonstration of a significant increase in iron after 48 hours of treatment. On the basis of the present data, however, it appears that the demands on enzyme systems involving iron and copper were not so great in the early stages of growth as on enzyme systems involving magnesium and manganese.

The exponential rate of increase for phosphorus in treated sections for the first 100 hours is, of course, directly related to the exponential increase in dry weight. In fact, the rate constants are about the same, i.e., 1% per hour.

Microscopic examination of treated stem sections showed that an increasing number of cells became meristematic during the period of the experiment. In spite of this qualitative difference between treated and untreated tissue and a marked difference in the amount of solid material involved per unit length of stem, the accumulation of calcium was not significantly different in treated and untreated stems. That meristematic tissue has a low calcium requirement is shown by the work of THODAY (13), who found a scarcity of calcium and an absence of calcium oxalate in the apical meristem of *Kleimia*. SCOTT (10) observed that the apical meristem of

Ricinus communis also was completely free of calcium oxalate crystals and commented: "It may be expected that microchemical analysis will indicate a lower and a higher calcium content in apical meristem and differential regions, respectively." CURRAN *et al.* (3) found that the calcium content of bacterial spore-formers was much lower in growing vegetative cells than in dormant spores.

Summary

1. Lanolin paste alone or lanolin paste containing 2% 3-indoleacetic acid was applied to the surface of middle portions of the first internodes of young bean plants (*Phaseolus vulgaris*) in a ring about 2 mm. wide.

2. Following this treatment, 1 cm. sections including the treated portions were removed at intervals of 1, 12, 30, 48, 72, 100, and 148 hours. Twenty to forty sections of both control and treated stems were taken at each interval to allow statistical determination of the significance of any differences that appeared.

3. Each section, after determination of fresh and dry weight, was analyzed spectrochemically for content of potassium, magnesium, calcium, phosphorus, iron, manganese, copper, aluminum, and boron. The data were expressed as micrograms per centimeter section. In addition, linear growth data were recorded and analyzed.

4. During the 6-day period, water in both control and treated stem sections increased linearly with time. The dry weight increases in untreated sections were slight and irregular; however, in the treated sections the dry weight increased exponentially with time.

5. Highly significant increases in potassium, magnesium, manganese, and boron in treated sections, as compared with the untreated ones, were first observed 30 hours after application. Potassium and magnesium in treated sections increased linearly with time, while changes in manganese and boron were irregular.

6. Phosphorus in the treated sections increased exponentially with time; the first significant increase was detected 48 hours following application. Copper also showed the first significant increase after 48 hours. The difference in iron content was certain only after 72 hours of treatment. Only in samples taken after 72 and 100 hours of treatment were there significantly greater amounts of aluminum in treated sections.

7. In both control and treated stem sections, calcium increased exponentially with time; the differences in amounts between treated and control stems were statistically insignificant.

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CONTRATOXIFICATION¹ OF PLANT GROWTH-REGULATORS IN SOILS AND ON PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 596

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Introduction

After plant growth-regulators have been applied to certain soils, in some instances they have persisted in them for so long a period that the cropping schedule has been seriously interfered with. CRAFTS (2) stated that planting of summer field crops in California was scarcely under way in 1945 before reports came in of failures on areas sprayed with 2,4-dichlorophenoxyacetic acid (2,4-D). In such cases methods of decreasing toxicity of the compounds in the soil, or complete elimination of toxicity, would be of importance.

It is often difficult to confine spray or dust applications of growth-regulators to the intended area. Sprays and dusts may drift considerable distances. A means of protecting the aerial parts of plants from the toxic effects of growth-regulators therefore would be of value.

¹ "Contratoxification" as used in this paper refers to the application of absorbents, adsorbents, or ion exchangers to soils or parts of plants in order to accomplish partial or complete elimination of the toxic effects of plant growth-regulators on plants.

Several investigators (3, 12) have demonstrated that various growth-regulators differ in degree of toxicity and in persistence of their toxicity in soils. Such studies are of importance, as the desired length of persistence of toxicity is dependent on the particular use to which a herbicide is put. For example, if soil sterilization for a long period is sought, then a growth-regulator of long persistence should be selected.

A recent study indicates that, when 2,4-D is added to soil several weeks before planting of a crop, stimulation of crop growth may result as well as a high degree of weed control (12). Such findings may result in an increased use of 2,4-D applied to soil before planting or before plants have emerged (pre-emergence treatments). For this use growth-regulators of high toxicity and of relatively short duration in the soil might be of great value.

The purpose of the experiments described in this paper was to devise means of decreasing or eliminating the toxic ef-

fects of plant growth-regulators applied to the soil or to the aerial parts of plants. The toxicity and persistence of toxicity of several growth-regulators under greenhouse and field conditions were also studied.

Persistence of growth-regulators in soil and their contratoxification

PERSISTENCE IN THE GREENHOUSE

MATERIAL AND METHODS.—The soil used was a 3:1 mixture by volume of a silt loam with coarse sand. The initial moisture content was about 18.2% and the pH value about 8.1. The moisture equivalent was 10.3% as determined by the centrifuge method.

The following compounds obtained from commercial sources were used for experimentation: 2,4-D, butyl ester of 2,4-D (BE 2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), O-isopropyl N-phenylcarbamate (IPPC), and copper 2,4-dichlorophenoxyacetate [Cu(2,4-D)₂]. The latter substance may have been a complex of several compounds but is hereafter arbitrarily referred to as Cu(2,4-D)₂.

Each of the five compounds in 6-gm. or 0.6-gm. quantities was separately dissolved in 100 ml. of ethyl alcohol. The solutions were then thoroughly stirred into 1-kg. lots of white quartz sand. On June 6, 1946, after the alcohol had evaporated, each batch of sand was thoroughly mixed with separate lots of soil of about 26.3 kg. each. Thus the growth-regulators were present in the soil at concentrations of 22 or 220 p.p.m., based on the dry weight of the soil. Quartz sand containing no growth-regulator was mixed with one lot of soil for a control.

About two-thirds of each lot was immediately stored in wooden boxes in a room, adjacent to the greenhouse, in

which no great temperature fluctuations occurred. Water was added occasionally to keep the soil in a moist condition. During the months of February, March, and April, 1947, however, it was air-dry.

The remaining one-third of each lot of soil was divided into ten aliquots. Each aliquot was placed in a 5-inch, white glazed pot of one-half gallon capacity, which had been provided with a glass-wool stopper over the drainage hole and one-fourth filled with coarse sand. Thus there were two concentrations of each of the five compounds and a control, each replicated ten times. The filled pots were placed on a greenhouse bench, and on June 6 five seeds of Red Kidney bean, ten of white mustard, and twelve of barley were planted in each crock.

On June 27 and November 16, 1946, and on May 7, 1947, aliquots of soil were removed from the wooden boxes and were similarly planted, except that there were only five replicates at the last planting. Plants of the May 7 planting were allowed to grow until September 2, 1947. At this time the old plants, which had produced fruit and then dried out, were pulled free from the soil, and the pots were replanted. This planting on September 2 was the fifth and final planting.

Rate and percentage of emergence of seedlings were used as primary criteria for judging the toxicity of the compounds in the soil, although further growth was also considered. The data were calculated in percentages, on the basis that controls equaled 100% (fig. 1). Emergence counts were made from 8 to 17 days after planting, depending on the weather conditions controlling the rate of growth. The pots were watered frequently to promote growth but not heavily enough to result in leaching.

OBSERVATIONS.—After the first planting, barley emerged in all pots except

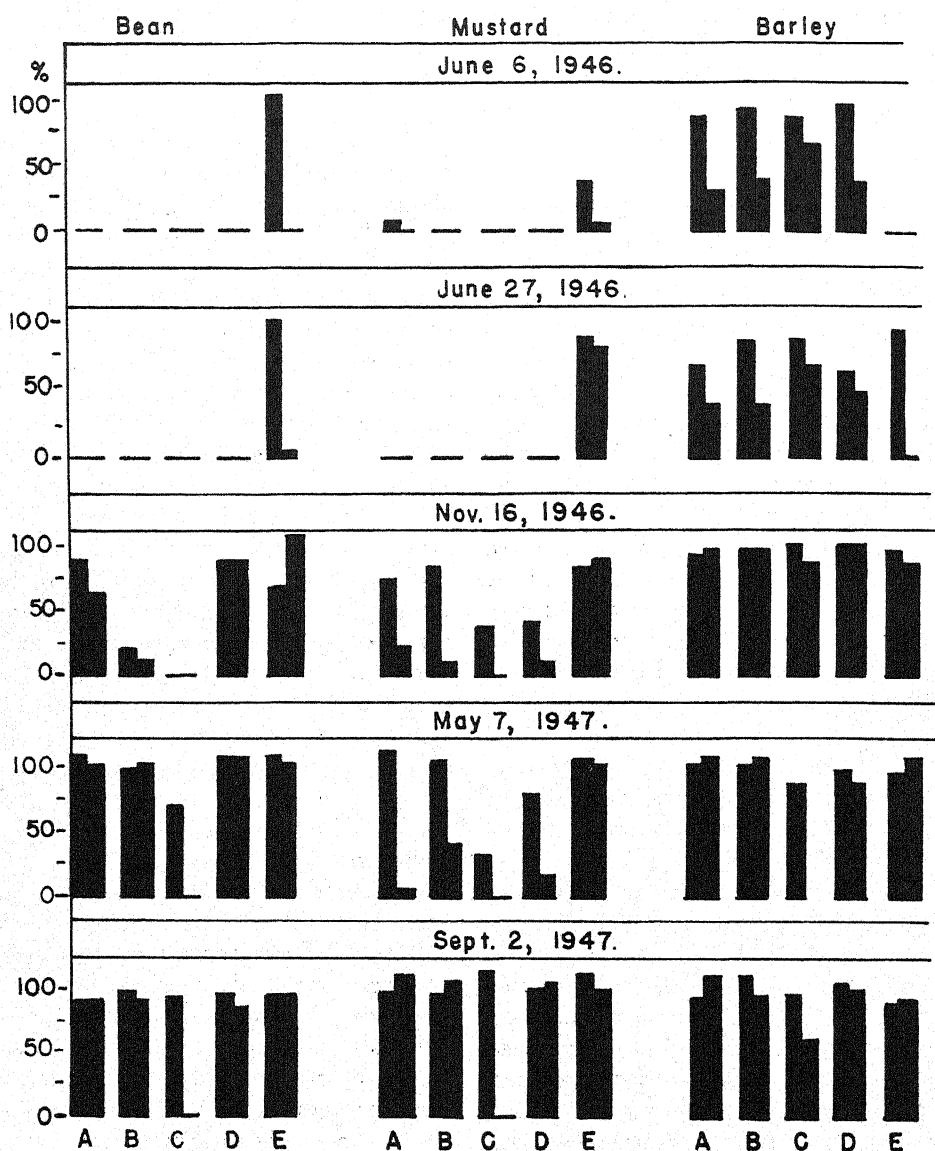


FIG. 1.—Percentages of emergence of test plants on indicated dates from treated and untreated soil stored since June 6, 1946. Germination of controls considered as 100%. Soil treatments: A, 2,4-D; B, BE 2,4-D; C, 2,4,5-T; D, Cu(2,4-D)₂; and E, IPPC. Left bar of each pair represents emergence from soil containing one compound at concentration of 22 p.p.m. and right bar at 220 p.p.m. Replanting of pots of fourth planting comprised final planting.

those containing IPPC. This situation was reversed in the case of Red Kidney bean and mustard (fig. 1). This difference in response of grasses and nongrasses to IPPC has been reported by several investigators (1, 12, 13).

Eleven days after the first planting, barley plants in all pots containing the low concentration of 2,4-D, $\text{Cu}(2,4\text{-D})_2$, BE 2,4-D, or 2,4,5-T were only about one-half as tall as controls—which were 3 inches in height—the leaf tips were browning, and root systems were small. The tops of barley plants in soil containing the high concentrations of the same compounds were merely white coleoptiles $\frac{1}{4}$ inch high. Ten days later all barley plants were dead and dried. Germination of mustard seeds in soil containing the low concentration of IPPC was much retarded compared with that of controls. Soil originally containing 22 p.p.m. of IPPC was the only treated lot in which normal kidney-bean plants developed at the first planting. Three weeks after planting, several small bean plants emerged from soil which originally contained 220 p.p.m. of IPPC. Their cotyledons came just above the soil surface, and the small primary leaves had less surface area than the cotyledons. The first internodes were only 3 or 4 mm. long.

Only the soil treated with IPPC showed a marked decrease in toxicity after 12 days of storage. All species in the second planting grew normally in soil originally containing 22 p.p.m. of IPPC.

Following the third planting seeds germinated slowly, and counts were not made until 17 days after planting. Barley grew normally in all soil except that originally treated with 220 p.p.m. of 2,4,5-T, in which the seedlings were much stunted. The growth of mustard plants indicated that the soil lot containing 2,4,5-T was more toxic than any of the

others, a condition not borne out by later experiments in the field. The soil containing $\text{Cu}(2,4\text{-D})_2$ was second highest in degree of toxicity (fig. 1). Germination and subsequent growth of beans from the planting of November 16, 1946, were extremely slow and uneven, probably because of low light intensities then prevalent in Chicago. It was evident, however, that 2,4,5-T was by far the most persistent of the five compounds studied.

During the period from November 16, 1946, to May 7, 1947, there was further decrease of toxicity in some of the soil lots. In the planting of May 7 Red Kidney beans which emerged from soil containing 2,4,5-T failed to produce normal root systems.

Between May 7 and September 2, 1947, toxic effects had disappeared in all lots except that treated with the high concentration of 2,4,5-T. In the latter soil barley coleoptiles $\frac{1}{2}$ inch in height showed above the surface 7 days after planting on September 2. Barley in other pots was then about 4 inches high.

PERSISTENCE IN THE FIELD

MATERIAL AND METHODS.—A lowland muck soil near Williams Bay, Wisconsin, was used. The surface few inches dried out rapidly after rains, although moist soil was always present at a depth of 2 or 3 inches. The moisture equivalent was 30.8% as determined by the centrifuge method, and the pH value about 8.0. Before treatment the soil was plowed, dragged, and raked in order to prepare a seed bed containing no large clods.

Thirty-one grams of 2,4-D, 35.8 gm. of 2,4,5-T, 35.3 gm. of $\text{Cu}(2,4\text{-D})_2$, or 38.9 gm. of BE 2,4-D were dissolved in ethyl alcohol. The solutions were thoroughly mixed with 1600-gm. amounts of white quartz sand, and then the alcohol was allowed to evaporate.

Fifteen plots 10×5 feet were laid out so that a randomized block design could be used. There were 5-foot border rows between plots. On June 21, 1947, 200 gm. of each of the sand-growth-regulator mixtures were scattered uniformly over separate plots. Each treatment was replicated three times, and three plots were left untreated as controls. The soil was lightly raked so that the sand was mixed with the upper $\frac{1}{2}$ inch. Thus plots were treated at a rate of 7.4 lb./acre of 2,4-D, 8.4 lb. of $\text{Cu}(2,4\text{-D})_2$, 8.5 lb. of 2,4,5-T, or 9.3 lb. of BE 2,4-D so that relatively equivalent amounts of the toxic principle of 2,4-D were applied.

Immediately after treatment one-fourth of each plot was planted with forty seeds of soybean and sixty-eight grains of Sudan grass, sowed in two 4-foot rows, 7 inches apart, of each type of seed. On July 3, 1947, a second planting was made, including one row of Red Kidney bean (twenty-five seeds) and one row of white mustard (fifty seeds) in addition to soybean and Sudan grass. Similar plantings were made on July 17, July 28, and August 5. All plantings were in the same one-half portion of each plot, which was possible, since later plantings were made after crops from early plantings had been harvested. Weeds were allowed to develop on the other half of each plot in order to observe the effectiveness of weed control.

Twelve to 22 days after planting, depending on the weather conditions controlling the rate of growth, emergence counts were made. Fresh weights of tops were obtained from 18 to 26 days after planting. At the time of harvest kidney beans and soybeans in control plots had usually expanded two trifoliate leaves, mustard plants were about 4 inches tall, and the average foliage height of Sudan grass was about 4 inches. The data were

calculated on a percentage basis and graphed (fig. 2).

On July 28, 37 days after the soil was treated, weeds in the unplanted portions of the plots were harvested, and the fresh weight of tops in an area 50×40 inches was obtained.

WEATHER.—Precipitation and temperature records were obtained from the Yerkes Observatory, Williams Bay, Wisconsin, which is about 2 miles south of the experimental area. Inches of precipitation in 1947 and mean monthly rainfall for a period of 29 years (shown in parentheses) were as follows: June, 4.67 (3.66); July, 3.33 (3.37); August, 1.40 (3.78); and September, 4.47 (3.83). Rainfall was normal during the latter part of June and during July, but there was a deficit of 2.38 inches below normal for August. From July 28 to August 29 only 0.65 inch of rain fell. During this period germination of seeds occurred very slowly.

The season was characterized by almost normal temperatures except during August, which had the highest mean temperature for this month in 29 years. Monthly temperatures (in °F.) were as follows, with the 29-year mean shown in parentheses: June, 64.2 (67.6); July, 70.3 (72.9); August, 78.1 (70.9); and September, 66.6 (63.5). The extremely high temperatures of August coincided with the drought during this period.

PERSISTENCE OF COMPOUNDS.—Sudan grass from the first planting in untreated plots had an average foliage height of about 10 inches at harvest time. In plots treated with 2,4,5-T it was about one-half as tall, and in the other treated plots the tops usually were merely reddish coleoptiles $\frac{1}{4}$ – $\frac{1}{2}$ inch long. Control soybeans were in the first or second trifoliate leaf stage and about $4\frac{1}{2}$ inches high at harvest time. A few soybeans in plots treated with 2,4,5-T grew normally, al-

though most failed to emerge. Stunted soybeans in plots treated with 2,4-D and $\text{Cu}(2,4\text{-D})_2$ were about 2 inches tall and had crinkled leaves with abnormal venation. The number of emerging plants and fresh weight of tops were calculated as percentages of the control values considered as 100% (fig. 2).

toxic compound to kidney beans. Plants in plots treated with 2,4-D, $\text{Cu}(2,4\text{-D})_2$, and BE 2,4-D were about one-half as tall as controls—which were 8 inches in height—and showed abnormal leaf development induced by the growth-regulators.

The development of plants from the

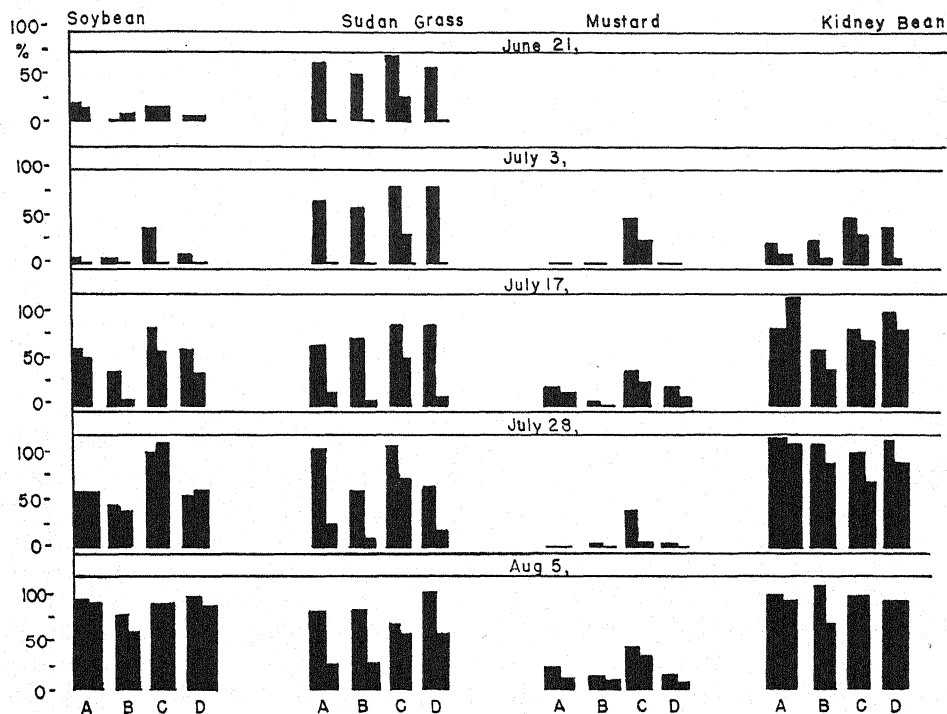


FIG. 2.—Percentages of emergence (*left bar of each pair*) and of fresh weights of tops (*right bar*) of plants growing in field soil previously treated with (A) 2,4-D, (B) BE 2,4-D, (C) 2,4,5-T, or (D) $\text{Cu}(2,4\text{-D})_2$. Percentages calculated on basis that controls equaled 100%.

Development of Sudan grass and soybeans from the second planting was similar to that from the first. In fact, the soil toxicity seemed greater at the second planting as judged by fresh weight of tops produced (fig. 2). Soil treated with 2,4,5-T was the least toxic; that treated with BE 2,4-D the most so. Red Kidney beans emerged in all plots, being much more tolerant of the growth-regulators than soybeans. 2,4,5-T was the least

third planting indicated that the toxicity of the growth-regulators had decreased between July 3 and July 17. The growth of soybeans showed soil treated with 2,4,5-T to be the least toxic, that with BE 2,4-D the most so. The fresh weight of tops of Sudan grass and the emergence and fresh weight of tops of mustard were confirmatory (fig. 2). Red Kidney beans developed well in all plots except those which had received applications of BE

2,4-D. Deformation of the leaves was much less at this planting than in the earlier ones.

The loss of toxicity from soil between July 17 and July 28 was small. Emergence and fresh weight of tops of soybean and Sudan grass were about the same in the fourth as in the third planting, except that plants in plots treated with 2,4,5-T made more growth from the fourth planting. There was little growth of mustard in treated plots except in those to which 2,4,5-T had been applied.

TABLE 1

FRESH WEIGHT (GM.) OF TOPS OF WEEDS 37 DAYS AFTER SOIL HAD RECEIVED SURFACE APPLICATIONS OF GROWTH-REGULATORS. AVERAGE OF THREE REPLICATE PLOTS

Treatment	Broad-leaved plants	Yellow bristle grass	Total
None.....	545.8	564.6	1110.4
2,4-D.....	200.7	105.1	305.8
Cu(2,4-D) ₂	161.1	27.6	188.7
2,4,5-T.....	439.1	365.1	804.2
BE 2,4-D.....	98.0	21.1	119.1

Drought and high temperatures during this period probably were associated with the small decrease in toxicity in the soil and with the poor plant growth. Kidney beans grew well. The primary leaves of soybeans and kidney beans in plots treated with 2,4-D, Cu(2,4-D)₂, or BE 2,4-D were abnormally veined and crinkled, but otherwise the plants appeared normal.

Data from the fifth planting in many cases showed larger values for emergence counts and fresh weight of tops than those of the fourth planting. Soybeans and kidney beans grew normally (except for crinkled primary leaves) in all treated plots except those treated with BE 2,4-D from which a lower percentage of emergence and fresh weight of tops were usually obtained. Growth of mustard

was much inhibited in all treated plots, although 2,4,5-T was the least toxic. Growth of Sudan grass was uneven, but there was indicated a decrease in toxicity in the soil treated with Cu(2,4-D)₂ as compared with that of the fourth planting.

WEED CONTROL.—On July 28, when weeds were harvested, control plots had a dense stand of weeds about 13 inches high. The principal species were: common purslane (*Portulaca oleracea* L.), yellow bristle grass (*Setaria lutescens* [Weigel] F. T. Hubb.), lamb's-quarter (*Chenopodium album* L.), galinsoga (*Galinsoga parviflora* Cav.), pigweed (*Amaranthus retroflexus* L.), and common chicory (*Cichorium intybus* L.). Yellow bristle grass, common purslane, and galinsoga were flowering. The application of 2,4,5-T resulted in only a small decrease in growth of weeds (table 1). The treatment with BE 2,4-D gave excellent control. Nevertheless, a few stunted plants of yellow bristle grass and galinsoga flowered. The density of weeds in plots treated with 2,4-D or Cu(2,4-D)₂ was only about one-fourth that of control plots, so that fair control was obtained. There were many plants of yellow bristle grass 2-4 inches high which had developed no normal root systems in the plots treated with 2,4-D or Cu(2,4-D)₂. These plants apparently absorbed water through the stubby roots at the crown which in many cases had formed tumors.

CONTRATOXIFICATION OF 2,4-D IN SOIL

Two types of experiments were performed in an attempt to eliminate or to decrease the toxicity of 2,4-D in soils. In the field, toxic soil was spaded (plowed), and in the field and greenhouse a cation exchanger, Zeo-Karb H, or an adsorbent, Norit A (activated charcoal), was mixed with it. The Zeo-Karb H was

obtained in granular form from the Permutit Company and the Norit A as a dust from the Pfanstiehl Chemical Company, Waukegan, Illinois. The high adsorptive capacities of Zeo-Karb H and Norit A for 2,4-D from aqueous solutions have been previously reported (7, 14).

SPADING.—On July 4 about 1700 gm. of sand-2,4-D mixtures were uniformly applied to each of four 18 × 18-foot plots adjacent to the field persistence experiment, at rates equivalent to 0, 10, 25, or 50 lb. of 2,4-D per acre. The sand was lightly raked into the upper $\frac{1}{2}$ inch. On July 10 a 6 × 3-foot portion of each area was spaded to a depth of about 6 inches. The soil was thus inverted as in plowing. The surface was then raked to make a seed bed. At the same time another 6 × 3 portion in each plot was left without spading but was raked lightly to produce a seed bed. Between the time of treatment with 2,4-D and spading or raking, 1.05 inches of rain fell. In each plot fifty seeds of soybean and of Red Kidney bean were uniformly planted at a depth of about $1\frac{1}{2}$ inches. On July 29 emergence counts were made, and on August 14, 35 days after planting, fresh weights of tops were obtained (table 2).

Spading (plowing) tended to decrease the toxicity of 2,4-D. At harvest time kidney beans in control plots had formed flower buds, and soybeans were flowering. Those in the spaded portion of the plot treated at the rate of 25 lb. of 2,4-D per acre appeared normal except for abnormal venation of their primary leaves. Those in the spaded plot treated with 50 lb./acre were severely stunted. These data conclusively show that the equivalent of spading (plowing) alone did in all cases at least lessen the toxic effects of 2,4-D. In unspaded soil the growth-regulator remained in the upper few inches, as is subsequently pointed out, and hence

spading probably decreased the concentration of the compound by placing some soil containing no growth-regulator at the surface.

The penetration of ammonium 2,4-dichlorophenoxyacetate [$\text{NH}_4(2,4\text{-D})$] applied at a rate of 10 lb./acre and of 2,4-D applied at three rates was determined. Six and 29 days after application a hole about $1\frac{1}{2}$ feet in diameter and 2 feet deep

TABLE 2

NUMBER OF PLANTS WHICH EMERGED FROM SPADED AND UNSPADED SOIL PREVIOUSLY TREATED WITH 2,4-D. FRESH WEIGHT (GM.) OF TOPS DETERMINED 35 DAYS AFTER PLANTING

TREATMENT (LB./ACRE OF 2,4-D)	KIDNEY BEAN		SOYBEAN	
	No. emerged	Weight of tops	No. emerged	Weight of tops
0 { Unspaded. . .	29	564.2	27	97.8
0 { Spaded.	28	611.5	33	196.5
10 { Unspaded. . .	23	165.4	18	11.1
10 { Spaded.	35	484.7	16	46.6
25 { Unspaded. . .	1	0	0	0
25 { Spaded.	23	183.6	7	0
50 { Unspaded. . .	0	0	0	0
50 { Spaded.	11	12.2	9	1.3

was dug in each treated area. Samples of soil were taken from the sides of the holes at depths of 0-3, 3-6, 6-9, 9-12, 12-18, and 18-24 inches. Each sample was mixed with one-third by volume of coarse sand and placed in two 2-inch, unglazed clay pots. About twenty-five seeds of white mustard were planted in each pot.

Character of germination of the seeds was the criterion of relative toxicity in the soil. The results showed that, with one exception, growth-regulators present in quantities toxic to mustard seedlings were confined to the upper 3 inches of soil. 2,4-D was also present in the 3-6-inch layer of soil in the plot treated at a

rate of 50 lb./acre. The relatively soluble ammonium salt failed to penetrate more deeply than the comparatively insoluble acid, which is in agreement with a previous study concerning degree and rate of leaching of several growth-regulating compounds (14).

FIELD APPLICATION OF ZEO-KARB H.—On July 4 a white quartz sand-2,4-D

control plot, and in a plot previously treated only with 2,4-D.

Emergence counts and fresh weight of tops of plants showed that Zeo-Karb H had eliminated toxic effects to plant growth. At the time of harvest, 33 days after planting, all plants were flowering except those in soil to which only 2,4-D had been added and from which seedlings

TABLE 3

EMERGENCE COUNTS AND FRESH WEIGHT (GM.) OF TOPS OF KIDNEY BEAN AND WHITE MUSTARD FROM SOIL PREVIOUSLY TREATED WITH 2,4-D. ZEO-KARB H OR NORIT A MIXED WITH SOIL 1 DAY BEFORE PLANTING. AVERAGES OF THREE REPLICATES

TREATMENT	AMOUNT OF CONTRA-TOXICANT USED		NO 2,4-D ADDED				2,4-D ADDED			
	P.p.m.	Lb./Acre*	Bean		Mustard		Bean		Mustard	
			No. emerged	Wt. of tops	No. emerged	Wt. of tops	No. emerged	Wt. of tops	No. emerged	Wt. of tops
Control....	0	0	4.3	8.2	11.3	0.43	0	0	0	0
Norit A....	81,252	49,520	4.7	12.1	8.7	0.43	4.3	8.4	9.0	0.35
	16,383	9,904	4.7	13.9	8.7	0.51	4.7	11.0	11.0	0.57
	8,192	4,952	4.7	10.8	10.3	0.70	3.7	9.3	11.3	0.52
	819	495	4.7	11.0	9.3	0.44	4.7	10.9	4.3	0.11
	218	132	5.0	8.7	11.0	0.58	3.7	6.5	0.3	0
Zeo-Karb H.	81,252	49,520	4.0	6.8	8.7	0.39	4.3	7.1	0	0.01
	16,383	9,904	5.0	8.9	8.0	0.30	1.3	2.6	0	0
	8,192	4,952	4.3	7.9	10.0	0.39	0.7	0	0	0
	819	495	4.3	7.7	13.00	0.58	0	0	0	0

* Approximate values calculated on basis that compounds were applied as surface application.

mixture was applied to a 5 × 15-foot plot on lowland soil at a rate equivalent to 10 lb. of 2,4-D per acre. Six days later 3500 gm. of Zeo-Karb H were uniformly applied to a 3 × 4-foot plot of the treated soil, and a similar quantity to an untreated plot. The ion exchanger was thoroughly mixed into the soil by raking to a depth of about 4 inches. On July 10 and 11 the areas were heavily watered. On July 12 twenty-five seeds of Red Kidney bean, twenty-five of soybean, and fifty of white mustard were uniformly planted in each of these plots, in an untreated

had failed to emerge. There were no weeds growing in the latter area, but, where Zeo-Karb H had eliminated the toxicity of 2,4-D, plants of common purslane covered most of the soil surface.

The data of this experiment clearly show that Zeo-Karb H will eliminate the toxic effects of 2,4-D in one field soil. The rates used, however, were far too great to be economically practicable.

GREENHOUSE EXPERIMENT WITH ZEO-KARB H AND NORIT A.—The purpose was to determine the effectiveness of small amounts of Zeo-Karb H and Norit

A in eliminating the toxic effects of 2,4-D in soil. A silt loam soil was mixed with coarse sand in the proportion of 3:1. The reaction of the mixture was about pH 8.0. A 0.39-gm. quantity of 2,4-D was dissolved in ethyl alcohol and the solution mixed with 1080 gm. of white quartz sand. After the alcohol was allowed to evaporate, the sand-2,4-D mixture was thoroughly incorporated with 15.4 kg. of the soil. The resulting mixture contained

after watering, five seeds of Red Kidney bean and fifteen of white mustard were planted in each pot. They were watered as necessary to provide conditions for growth.

On September 14, 9 days after planting, emergence counts were made, and fresh weight of tops obtained (table 3, fig. 3). Plants in all pots containing no 2,4-D grew normally. Norit A was very effective in eliminating the toxic effects

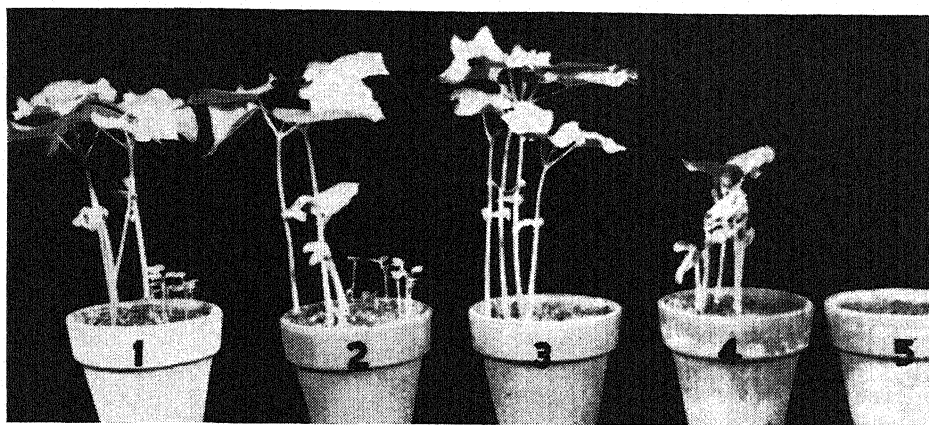


FIG. 3.—Growth of kidney beans and white mustard in soil treated with 24 p.p.m. of 2,4-D. Control soil at left; other pots (left to right) also contained 8,192, 819, 218, or 0 p.p.m. of Norit A mixed with soil 1 day before planting. Lowest concentration of Norit A decreased toxicity of soil. Photo taken 8 days after planting.

2,4-D at a rate of about 24 p.p.m. when calculated on an air-dry basis. A similar lot of control soil was thoroughly mixed with 1080 gm. of sand. Each of the two lots of soil was then divided into ten equal aliquots. To aliquots from each lot were added 135, 27, 13.5, or 1.35 gm. of Zeo-Karb H or Norit A. To another aliquot from each lot was added 0.36 gm. of Norit A. The tenth aliquot received no Zeo-Karb H or Norit A. After thorough mixing, each aliquot was placed in three replicate 4-inch unglazed clay pots on a greenhouse bench. They were then watered to make it possible for the 2,4-D to diffuse in the soil; whether it did so is problematical. On September 5, 21 hours

of 2,4-D in the soil. Kidney beans germinated in soil previously treated with 2,4-D to which 218 p.p.m. of Norit A had also been added, although their emergence was delayed for several days. Some mustard seeds germinated in soil treated with 2,4-D to which as little as 819 p.p.m. of Norit A had been added, and the seedlings grew normally in soil to which larger quantities of the latter had been applied. There is no doubt that the elimination of toxicity was brought about by Norit A, since seeds were planted within 1 day after application of 2,4-D. Several days are usually required for other types of inactivation of 2,4-D in soil.

Zeo-Karb H was much less effective

than Norit A in rendering 2,4-D non-toxic. All plants growing in soil containing 2,4-D and Zeo-Karb H were stunted and showed crinkling of leaves and abnormal venation, indicating some absorption of 2,4-D into the plants. Even the high concentration of 81,252 p.p.m. of Zeo-Karb H failed to eliminate completely the toxic effects, as primary leaves of kidney beans in these pots were abnormally veined. It is probable that the effectiveness of Zeo-Karb H would be greatly increased if it were ground to a fine dust before use.

Contratoxification on aerial parts of plants

MATERIAL AND METHODS

The soil used consisted of one part fine gravel, one part decomposed leaf mold, and two parts by volume of a heavy loam soil, placed in 4½-inch unglazed clay pots. They were planted with Red Kidney bean, soybean, white mustard, or marigold. After emergence the plants were thinned so that there were two of kidney bean, three of soybean, and about fifteen of white mustard or marigold per pot. In two experiments kidney beans were grown in flats.

The following growth-regulators were obtained from commercial sources:

Ammonium 2,4-dichlorophenoxyacetate
[NH₄(2,4-D)]

Butyl ester of 2,4-D (BE 2,4-D)

Sodium 2,4-dichlorophenoxyacetate
[Na(2,4-D)]

Triethanolamine salt of 2,4-D (TEA 2,4-D)

Weed-No-More agricultural dust (BE 2,4-D dust)

Isopropyl ester of 2,4-D (IE 2,4-D)

The BE 2,4-D dust contained 5% BE 2,4-D, 20% oil, and inert material and was applied using a cheesecloth bag. Aqueous solutions of 0.1% were made of the other compounds and were sprayed

on the plants with a DeVilbiss spray gun at a pressure of 15 lb. The plants were sprayed heavily but not to the extent that liquid dripped from the leaves.

Norit A (activated charcoal) or Zeo-Karb H was usually used to eliminate or lessen the toxicity of the growth-regulators, although the effectiveness of bone black, lamp black, and several types of clay was also tested. Before use the Zeo-Karb H was ground in a micropulverizer and passed through a slotted screen with slit-width of 0.013 inch. Norit A, Zeo-Karb H, and certain other substances are hereafter referred to as "contratoxicants." They were applied as water suspensions using a DeVilbiss spray gun or as dusts using a hand duster or cheesecloth bags.

All treatments were replicated three or four times unless otherwise indicated.

APPLICATION OF CONTRATOXICANTS BEFORE TREATMENT WITH GROWTH-REGULATORS

NORIT A.—In one experiment Red Kidney bean, soybean, and marigold were used. At the time of treatment on November 18, 1947, kidney beans were 7 inches in height and had expanded their first trifoliate leaves; soybeans were about 6½ inches tall, with their first trifoliate leaves just beginning to expand; and marigolds were 2 inches high, with two true leaves. A thin film of Norit A was applied to some of the plants with a hand duster immediately after a spray of water had been applied. The film of water aided in sticking the dust to the leaves.

At 11:00 A.M. some dusted and undusted kidney beans were sprayed with 0.1% solutions of NH₄(2,4-D), BE 2,4-D, or IE 2,4-D. Kidney beans with and without previous applications of contratoxicant were also dusted with BE 2,4-D dust. One lot of plants received applica-

tions of contratotoxicant only, and another was untreated and served as controls. Soybeans were similarly treated with BE 2,4-D or $\text{NH}_4(2,4\text{-D})$, and marigolds with BE 2,4-D or TEA 2,4-D. Each treatment was replicated three times. The day was partly cloudy.

All kidney beans treated only with growth-regulators exhibited much shoot curvature and crinkling of primary leaves 27 hours after treatment. These symptoms were very slight, however, in plants protected with contratotoxicant. Twenty

The contratotoxicant also protected soybeans against growth-regulators (table 4, fig. 5). At the time of harvest control plants and those treated only with Norit A were 9 inches tall, whereas plants which received growth-regulator only were dying or already dead and dried.

Norit A gave much protection to marigolds sprayed with growth-regulators (table 4). At the time of harvest control plants were $4\frac{1}{2}$ inches tall, and those treated with contratotoxicant only were 4 inches.

TABLE 4

FRESH WEIGHT (GM.) OF TOPS OF KIDNEY BEAN, SOYBEAN, AND MARIGOLD 22 DAYS AFTER TREATMENT. AVERAGES OF THREE REPLICATES

TREATMENT	KIDNEY BEAN		SOYBEAN		MARIGOLD	
	With Norit A	Without Norit A	With Norit A	Without Norit A	With Norit A	Without Norit A
Control.....	8.15	9.42	4.57	3.97	1.60	2.13
IE 2,4-D.....	5.90	1.00*
BE 2,4-D.....	7.10	0.83*	4.17	0.67*	0.52	0.05*
$\text{NH}_4(2,4\text{-D})$	6.97	6.07†	4.03	3.43†
BE 2,4-D dust.....	8.30	3.67
TEA 2,4-D.....	0.59	0.00*

* Dead.

† Yellowing and dying.

days after treatment, control kidney beans were 9 inches high and flowering. Plants which received only contratotoxicant were slightly smaller than controls, and their leaves showed a small amount of injury. Kidney beans treated only with growth-regulators were dead, except those sprayed with $\text{NH}_4(2,4\text{-D})$ which were yellowing and dying. Norit A gave much protection against all applications of the growth-regulators. Some plants treated with both contratotoxicant and growth-regulator were somewhat stunted and showed some stem swelling, abnormal leaf venation, etc., but, in general, most of them had grown almost normally (table 4, fig. 4).

In a second experiment Red Kidney bean, soybean, mustard, and marigold were used. Kidney beans were 7 inches tall but had not expanded their first trifoliate leaf. Soybeans were 8 inches, and mustard and marigold $1\frac{1}{2}$ inches in height. The two latter species had two true leaves each. Some plants of each kind were sprayed at 11:00 A.M., November 12, with a 20% aqueous suspension of Norit A. About 15 minutes later kidney beans with, and some without, Norit A were sprayed with 0.1% solutions of $\text{Na}(2,4\text{-D})$, $\text{NH}_4(2,4\text{-D})$, BE 2,4-D, or TEA 2,4-D, or were dusted with BE 2,4-D dust. Some plants were treated

with contratoxicant only, and others were defoliated.

A similar experiment with soybeans and mustard was set up using BE 2,4-D or $\text{NH}_4(2,4\text{-D})$, and one with marigold using $\text{NH}_4(2,4\text{-D})$.

Twenty hours after treatment the primary leaves of young kidney beans treated with Norit A, with or without subsequent application of growth-regulator, were wilted, and 24 days later portions of these leaves were dried. A new trifoliate leaf had, however, developed.

These results show that Norit A is somewhat toxic to very young Red Kidney beans.

On December 7 control kidney beans were 10 inches tall and flowering. Beans treated with growth-regulators only had enlarged stems, tumors at the terminal growing points, etc., but those protected by Norit A before being sprayed with growth-regulators showed none of the typical responses to the latter compounds. Less protection was obtained against BE 2,4-D dust than against the



FIG. 4.—Kidney beans 20 days after treatment with Norit A and/or growth-regulators. *A*, left to right: (1) control, (2) Norit A, (3) Norit A—then IE 2,4-D, (4) IE 2,4-D, (5) Norit A—then BE 2,4-D, and (6) BE 2,4-D. *B*, left to right: (1) control, (2) Norit A, (3) Norit A—then BE 2,4-D dust, (4) BE 2,4-D dust, (5) Norit A—then $\text{NH}_4(2,4\text{-D})$, and (6) $\text{NH}_4(2,4\text{-D})$. Norit A protected plants against all growth-regulators used.

sprays. The defoliated plants were dead at harvest time. Their failure to recover was indicative of the lack of vigor during the season of low light intensity.

In this experiment Norit A afforded only slight protection to soybeans against growth-regulators. The suspension tended to coalesce in large droplets on their hairy leaves so that the contratoxicant was unevenly distributed. Dusting is probably one of the effective methods of

obtaining uniform coverage on hirsute leaves or on those not easily wetted.

On December 10 control plants of marigold were 4 inches tall and had four true leaves each; those treated only with Norit A were 3 inches tall. The contratoxicant provided much protection to marigolds against $\text{NH}_4(2,4\text{-D})$ and gave partial protection to white mustard against the growth-regulators (table 5, fig. 6).



FIGS. 5, 6.—Fig. 5 (*above*), soybeans 20 days after treatment with Norit A and/or growth-regulators. *Left to right*: (1) control, (2) Norit A, (3) Norit A—then $\text{NH}_4(2,4\text{-D})$, (4) $\text{NH}_4(2,4\text{-D})$, (5) Norit A—then BE 2,4-D, and (6) BE 2,4-D. Contratoxicant eliminated toxic effects of growth-regulators. Fig. 6 (*below*), marigolds 28 days after treatment with Norit A and/or $\text{NH}_4(2,4\text{-D})$. *Left to right*: (1) control, (2) Norit A, (3) $\text{NH}_4(2,4\text{-D})$, and (4) Norit A—then $\text{NH}_4(2,4\text{-D})$. Contratoxicant gave much protection against $\text{NH}_4(2,4\text{-D})$.

ZEO-KARB H.—Red Kidney beans with their first trifoliate leaf expanded and soybeans just beginning to expand a similar leaf were used. Some plants were sprayed with a 20% aqueous suspension of Zeo-Karb H so that the leaves ap-

TABLE 5

FRESH WEIGHT (GM.) OF TOPS OF MARIGOLD AND WHITE MUSTARD 28 DAYS AFTER TREATMENT. AVERAGES OF FOUR REPLICATES

TREATMENT	MARIGOLD		MUSTARD	
	With Norit A	Without Norit A	With Norit A	Without Norit A
Control . . .	1.83	3.28	1.64	3.73
NH ₄ (2,4-D) .	2.68	0.33	0.63	0.15
BE 2,4-D . . .			1.59	00

peared well blackened. Ten minutes later untreated kidney beans and some treated with contratoxicant were given applications of Na(2,4-D), BE 2,4-D, TEA 2,4-D, IE 2,4-D, or BE 2,4-D dust. With soybeans, BE 2,4-D only was used.

After 20 hours kidney beans treated only with growth-regulators exhibited much epinasty, shoot curvature, and crumpling of leaves, but those previously treated with Zeo-Karb H showed only slight indications of injury. On December 8 control kidney beans were 9 inches tall and flowering; plants treated only with contratoxicant were slightly smaller, and those sprayed only with growth-regulators were usually dead. Zeo-Karb H gave almost complete protection against four of the growth-regulators; only partial protection was obtained against BE 2,4-D dust (table 6, fig. 7).

The contratoxicant gave little protection to soybeans against BE 2,4-D, probably because the former was applied in aqueous suspension instead of as dust.

Another experiment was performed to determine the effectiveness of a dust

application of Zeo-Karb H in protecting soybeans against BE 2,4-D sprays. Some soybeans on which the first trifoliate leaves were beginning to expand were dusted with contratoxicant at 10:00 A.M. on November 24, a cloudy day. Immediately afterward some dusted and undusted plants were sprayed with a 0.1% solution of BE 2,4-D. Two weeks later those which had received BE 2,4-D only were dead and dried, but all others had grown normally (fig. 8). This experiment was repeated using kidney beans, and similar results were obtained.

OTHER COMPOUNDS.—Another experiment tested the effectiveness as contratoxicants of 20% aqueous suspensions of attapulugus clays, super filtrol (a filtering material for decolorizing and purifying), and bone black, and of a 3.3% suspension of lamp black. A 20% suspension of the latter proved too thick to spray. A similar set of suspensions was prepared containing, in addition, 0.9% of the base mixture of commercial Weed-No-More. This base mixture contained no growth-

TABLE 6

FRESH WEIGHT (GM.) OF TOPS OF KIDNEY BEANS 18 DAYS AFTER TREATMENT AVERAGES OF THREE REPLICATES

Treatment	With Zeo-Karb H	Without Zeo-Karb H
Control	5.82	7.73
Na 2,4-D	8.52	4.45*
BE 2,4-D	6.97	0.83
TEA 2,4-D	6.72	0.77
IE 2,4-D	6.85	0.83
BE 2,4-D dust	5.87	1.40

* Plants yellowing and dying.

regulator and was used as a wetting agent. Many growth-regulators can be formulated with it.

Red Kidney beans 11 inches tall, with their first trifoliate leaves beginning to expand, were used as test plants. Flats of

them were sprayed in triplicate with one of each of the suspensions on a cloudy day. One flat of each set was sprayed about 2 hours later with a 0.1% solution of $\text{NH}_4(2,4\text{-D})$, and another with 0.1% BE 2,4-D. The third was left without further treatment. Some plants received $\text{NH}_4(2,4\text{-D})$ or BE 2,4-D only, without previous treatment. Others were untreated and served as controls.

Thirteen days later control plants were 12 inches tall and had two or three tri-

foliate leaves (fig. 9). Plants sprayed only with growth-regulators were yellowing and dying. None of the clays furnished any protection against the growth-regulators (fig. 9).

Suspensions of bone black and lamp black containing no base mixture were very toxic to the plants; the primary leaves were partly dried out, and growth was greatly retarded; some plants died. When base mixture was present, the toxicity was greatly lessened (fig. 10).



FIG. 7.—Kidney beans 20 days after treatment with Zeo-Karb H and/or growth-regulators. *A*, left to right: (1) control, (2) Zeo-Karb H, (3) Zeo-Karb H—then TEA 2,4-D, (4) TEA 2,4-D, (5) Zeo-Karb H—then IE 2,4-D, and (6) IE 2,4-D. *B*, left to right: (1) control, (2) Zeo-Karb H, (3) Zeo-Karb H—then BE 2,4-D, (4) BE 2,4-D, (5) Zeo-Karb H—then BE 2,4-D dust, and (6) BE 2,4-D dust. In all cases Zeo-Karb H provided almost complete protection.

Suspensions of lamp black, without base mixture, provided some protection against the growth-regulators, and bone black, without base mixture, gave considerable protection even though these two substances in themselves caused much contact damage. The addition of base mixture greatly decreased the effectiveness of the contratoxicants, although

base mixture were little injured by $\text{NH}_4(2,4\text{-D})$, but, when base mixture had been added, little or no protection was afforded (fig. 11). When larger quantities of contratoxicants were applied, however, almost complete protection against the growth-regulator was obtained, even though base mixture had been added to the contratoxicant.

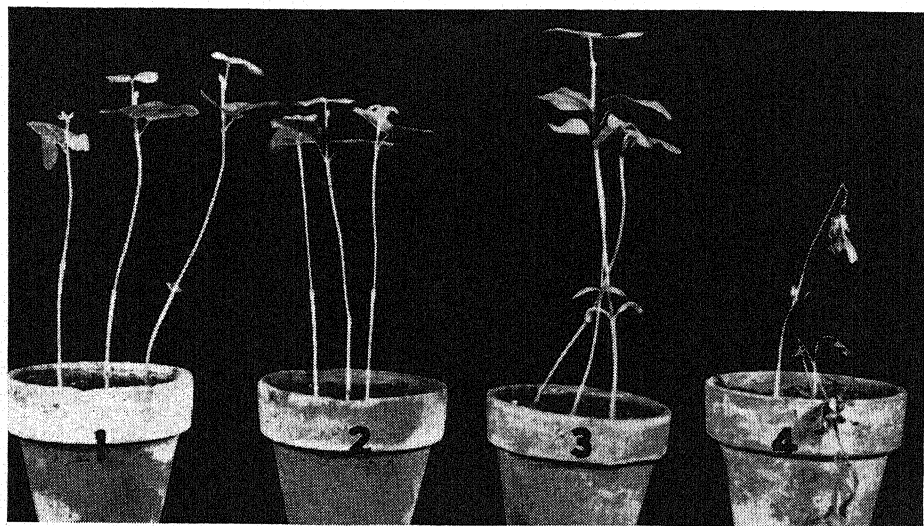


FIG. 8.—Soybeans 12 days after treatment with Zeo-Karb H and/or BE 2,4-D. *Left to right:* (1) control, (2) Zeo-Karb H, (3) Zeo-Karb H—then BE 2,4-D, and (4) BE 2,4-D. Contratoxicant provided complete protection to soybeans.

the suspension of bone black with base mixture provided much protection against $\text{NH}_4(2,4\text{-D})$ (fig. 9).

The decrease in effectiveness of contratoxicants when mixed with base mixture was brought out in another experiment in which young kidney beans were sprayed with 1% suspensions of Norit A or 5% of Zeo-Karb H. Other plants were sprayed with similar suspensions containing 0.9% of base mixture. Some plants of each set were sprayed 15 minutes later with 0.1% $\text{NH}_4(2,4\text{-D})$. Plants protected by the contratoxicants containing no

APPLICATION OF CONTRATOXICANTS AFTER SPRAYING WITH GROWTH-REGULATORS

NORIT A.—The purpose was to determine whether Norit A could prevent toxicity symptoms of BE 2,4-D when applied to plants previously treated with BE 2,4-D. Kidney beans just beginning to expand their first trifoliate leaves were used. At 1:00 P.M. on November 14, an overcast day, plants were sprayed with a 0.1% solution of BE 2,4-D. Fifteen minutes and 1, 6, 12, 24, or 48 hours later they were sprayed with a 5% aqueous

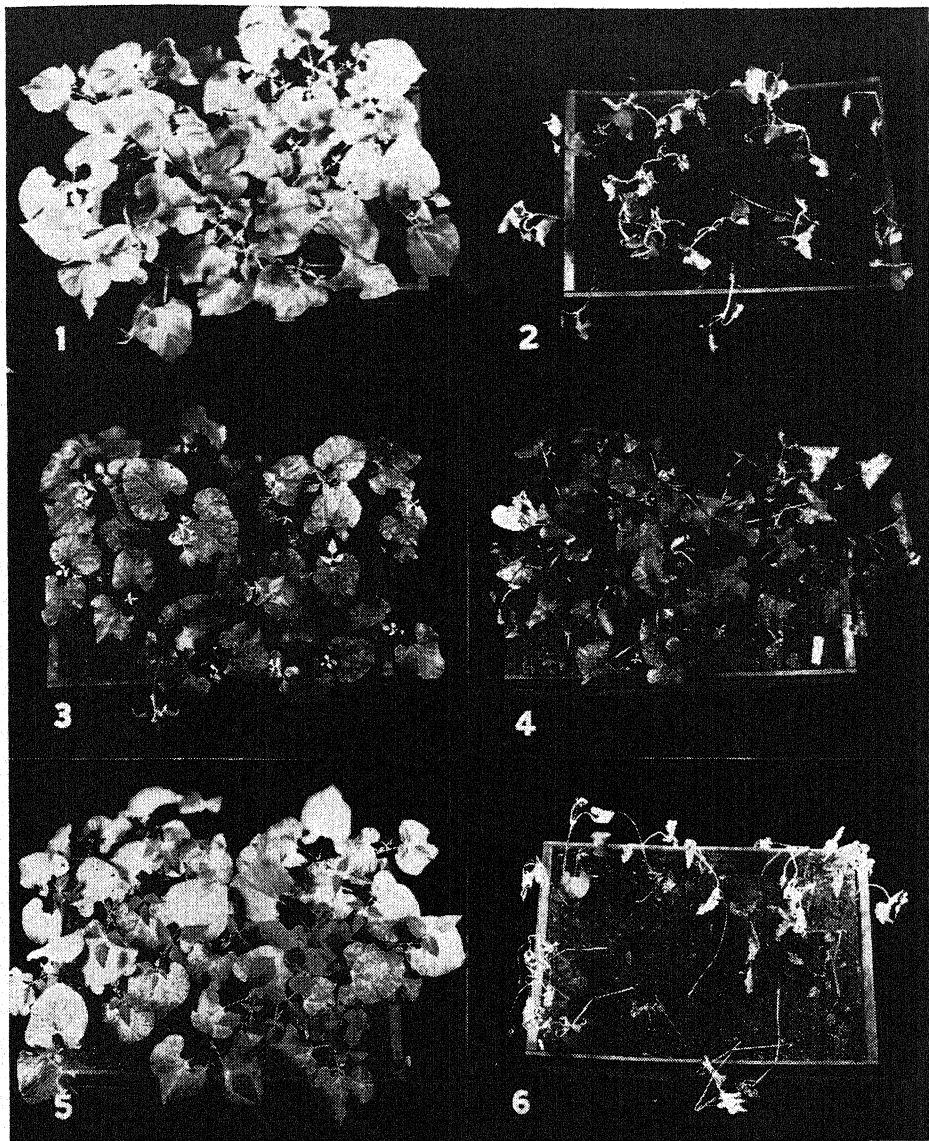


FIG. 9.—Kidney beans 13 days after spraying with bone black or super filtrol and/or 0.1% $\text{NH}_4(2,4\text{-D})$. (1) untreated control, (2) $\text{NH}_4(2,4\text{-D})$ only, (3) bone black with base mixture only, (4) bone black with base mixture—then $\text{NH}_4(2,4\text{-D})$, (5) super filtrol with base mixture only, and (6) super filtrol with base mixture—then $\text{NH}_4(2,4\text{-D})$. Bone black provided partial protection, but super filtrol furnished none.

suspension of Norit A. The upper leaf surfaces were in each case wet with the suspension even though the stems had already bent over as a result of the previous BE 2,4-D application. One set of plants sprayed with growth-regulator received no contratotoxicant, and another received only contratotoxicant.

Twenty-four days after treatment control plants were flowering, and those which had received applications of Norit A only showed injury of the pri-

mary leaves. The latter plants had, however, made much new growth. Plants sprayed with Norit A suspension 15 minutes after treatment with BE 2,4-D were alive, although they exhibited stem enlargement and tumors. All plants sprayed with Norit A suspension more than 15 minutes after BE 2,4-D had been applied were dead and dried (table 7). These results would indicate that the contratotoxicant should be applied within 15 minutes after treatment with a growth-regulator formulated with oil to affect markedly plant responses to the regulator.

ZEO-KARB H.—This experiment was repeated on November 24, except that a

VARIOUS RATES OF CONTRATOXICANTS

NORIT A.—An experiment was performed to determine how much Norit A is necessary to protect plants from a

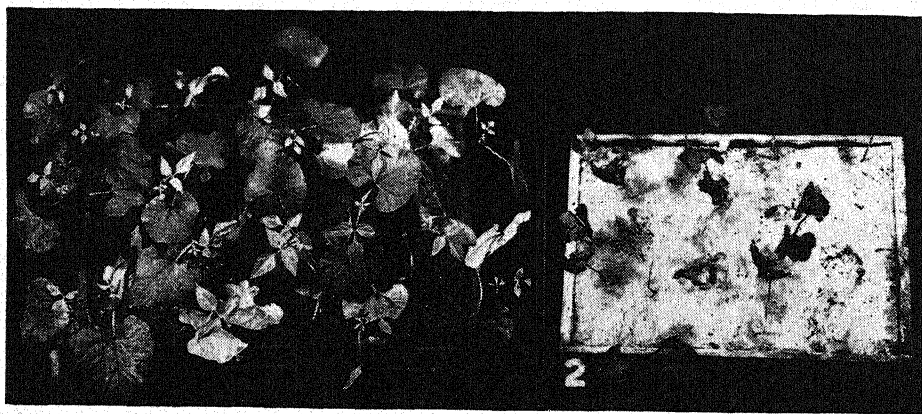


FIG. 10.—Kidney beans 13 days after treatment with suspension of lamp black containing base mixture (1) or without base mixture (2). Suspension of lamp black without base mixture is very toxic, but, when base mixture was present, little injury was produced.

0.1% aqueous spray of $\text{NH}_4(2,4\text{-D})$. Red Kidney beans beginning to expand their first trifoliate leaves were used. At 2:00 P.M. on November 13, a sunny day, plants were heavily sprayed with 20, 5, 1, 0.1, or 0.01% aqueous suspensions of Norit A, at rates equivalent to about 600, 75, 15, 1.5, or 0.15 lb. of Norit A per acre. About 10 minutes later some plants from each lot were sprayed with 0.1% aqueous solution of $\text{NH}_4(2,4\text{-D})$; others were treated with $\text{NH}_4(2,4\text{-D})$ only; and others were untreated.

Twenty-four hours later plants which had been sprayed with a suspension of contratotoxicant of 5% concentration or greater and then with $\text{NH}_4(2,4\text{-D})$



FIG. 11.—Kidney beans 9 days after spraying with aqueous 1% suspension of Norit A and/or growth-regulator. (1) Control, (2) $\text{NH}_4(2,4\text{-D})$ only, (3) Norit A, (4) Norit A with 0.9% base mixture, (5) Norit A—then 0.1% $\text{NH}_4(2,4\text{-D})$, and (6) Norit A with 0.9% base mixture—then $\text{NH}_4(2,4\text{-D})$. Base mixture made contratoxicant ineffective.

showed little epinasty or shoot curvature such as was manifested by plants sprayed with a lower concentration of Norit A and then with $\text{NH}_4(2,4\text{-D})$ or with $\text{NH}_4(2,4\text{-D})$ only. Twenty-five days after treatment controls were flowering. Plants sprayed only with 20% or 5% suspensions of Norit A were injured, but a new trifoliate leaf had been produced. Little or no stem enlargement and few or no tumors were found on plants

TABLE 7

FRESH WEIGHT (GM.) OF TOPS OF KIDNEY BEANS TREATED WITH CONTRATOXICANTS AT VARYING INTERVALS AFTER SPRAYING WITH BE 2,4-D; 24 AND 16 DAYS AFTER TREATMENT WITH NORIT A AND ZEO-KARB H, RESPECTIVELY. AVERAGES OF THREE REPLICATES

Time of application of contratoxicant after spraying with BE 2,4-D	Norit A—then BE 2,4-D	Zeo-Karb H—then BE 2,4-D
15 min.....	5.4	4.7
1 hr.....	0.6*	2.7
6 hr.....	0.6*	0.8*
12 hr.....	0.5*	0.7*
24 hr.....	0.5*	0.8*
48 hr.....	0.6*	0.7*
No contratoxicant..	0.6*	0.7*
No BE 2,4-D	4.4	6.3
Control.....	10.1	7.4

* Dead and dried.

treated with $\text{NH}_4(2,4\text{-D})$ previously sprayed with 20% or 5% suspensions of contratoxicant. When lesser amounts of contratoxicant were used, however, symptoms typically resulting from growth-regulators occurred. Thus, an amount of Norit A between 15 and 75 lb./acre would be needed to protect Red Kidney beans against aqueous sprays of $\text{NH}_4(2,4\text{-D})$ under the conditions of this experiment.

ZEO-KARB H.—Red Kidney beans 7 inches tall with the first trifoliate leaf expanded were used. At 3:00 P.M. on November 22, a sunny day, they were

sprayed with 20, 5, or 1% aqueous suspensions of Zeo-Karb H. Ten minutes later some plants from each lot were sprayed with a 0.1% solution of BE 2,4-D. Other plants received BE 2,4-D only.

Sixteen days later controls and plants treated only with Zeo-Karb H were flowering; plants sprayed only with BE 2,4-D were dead and dried. The 20% suspension of contratoxicant gave excellent protection against the growth-regulator, 5% gave much, but the 1% suspension gave little or none (table 8).

TABLE 8

FRESH WEIGHT (GM.) OF TOPS OF KIDNEY BEAN TREATED WITH ZEO-KARB H AND/OR 0.1% BE 2,4-D. GROWTH-REGULATOR APPLIED AFTER CONTRATOXICANT. HARVESTED 16 DAYS AFTER TREATMENT. AVERAGES OF THREE REPLICATES

Treatment	No BE 2,4-D applied	BE 2,4-D applied
20% Zeo-Karb H.....	8.45	7.43
5% ".....	7.77	4.33
1% ".....	9.40	2.97*
no.....	9.41	1.20*

* Dead.

This experiment was repeated using $\text{NH}_4(2,4\text{-D})$, and the results were in agreement.

A greater quantity of Zeo-Karb H than of Norit A, when used in aqueous suspension, was necessary for protection of plants against sprays of growth-regulators. The particle size of Zeo-Karb H was larger, however, than that of Norit A, and it is probable that effectiveness of contratoxicants is greater in the case of smaller particle sizes.

Discussion

The data of DeRose (3), who studied the relative rates of disappearance of several compounds from soil, are in general agreement with the results of the

persistence experiment in the greenhouse. In the field experiment here reported 2,4,5-T was the least toxic compound used and the toxicity also persisted a shorter period. This is contradictory to the results of the greenhouse experiment and to the work of DE ROSE. It may be that the amount of 2,4,5-T used in the field, 8.5 lb./acre, was too little effective-ly to sterilize the soil; a greater amount might have been much more persistent as well as more toxic. The relatively short persistence of 2,4,5-T in the field may have been associated with the type and quantity of organic matter present in the soil; the amount in the field soil used was very high (4, 6).

TAYLOR (12) found that the effects of treatment with 4 lb./acre of 2,4-D and IPPC appeared to persist for about 7 and 5 weeks, respectively, in two neutral or nonacid loam soils in Wisconsin. It is significant that rainfall was above normal during the period of his experiment.

With the exception of the BE 2,4-D treatment in the field, soils treated with BE 2,4-D, 2,4-D, and $\text{Cu}(2,4\text{-D})_2$ lost their toxicity generally at about the same rate. HANKS (5) demonstrated that 2,4-D and calcium 2,4-dichlorophenoxy-acetate were leached from each of six widely different soils at about equal rates, as measured by tests of toxicities of leachates, using a modification of the corn-root bio-assay method of SWANSON (11).

BE 2,4-D was the most toxic and persistent of the compounds tested in the field. Perhaps it is chemically more stable than the other compounds used. Another factor may be a greater capacity of the ester to permeate the soil both horizontally and vertically. These factors may have been associated with the high degree of weed control in plots treated with BE 2,4-D.

The great decrease in toxicity in the cropped soil of the greenhouse experiment which was replanted on September 2, 1947, is in agreement with the results of KRIES (6). The more rapid disappearance of toxic effects from cropped soil than from soil in storage probably resulted from factors other than the presence of growing plants only. Water was added much more frequently to the cropped soil, the temperature and degree of aeration were different, and both chemical and biological activity may have been greater in it.

The greater growth of soybeans in the field in the treated plots following the first planting as compared with the second may have been associated with the length of time between planting and the first heavy rainfall following planting. There was 0.06 inch of rain during an 8-day period following the first planting. Although soybeans began to germinate during this dry period, they grew slowly. Three days after the second planting 1.04 inches of rain fell. It thus appears that rainfall tended to increase the toxic effects of the herbicidal growth-regulator in the soil. It is also possible that water or water vapor in a dry soil could reach the seeds, making germination possible, while there might be little or no movement of the growth-regulator in the soil. There also may be an association between vegetative activity of plants and their response to growth-regulators. Plants with ample water grew rapidly but were more severely injured by growth-regulators than plants under drier conditions that exhibited less vegetative activity.

The disappearance of toxic effects of growth-regulators was apparently closely associated with rainfall. From June 21 to July 3, only the rain of 1.02 inches on June 29 was of any magnitude. During

this period there was little or no loss of toxicity from the soil as reflected by growth of soybeans and Sudan grass. From July 3 to July 17 there were 2.09 inches of rain, heavy showers falling on July 6 and 13. Great decrease of toxicity occurred during this period as shown by results of the third planting. Further gradual reduction in toxicity occurred between July 17 and August 5. During this period there were 1.44 inches of precipitation. On August 5, 45 days after application of the growth-regulators, the soil was still toxic, an over-all result of the dry experimental period. If a crop sensitive to the growth-regulators had been planted at the end of this interval, its yield probably would have been low.

Several investigators have shown that the toxic effects of 2,4-D disappear far more rapidly in moist than in dry soil (6, 8). CRAFTS (2) has urged special caution in the use of 2,4-D in semiarid regions, where the compounds persist for a long time in the soil. KRIES (6) and HANKS (5) have pointed out that 2,4-D may persist longer in soils of some alkalinity.

In the field experiments 2,4-D remained in the upper soil layers, the compound perhaps having been adsorbed there (7, 14). In many soils it might leach deeply as indicated by greenhouse experiments (3, 5, 9). In such cases plowing would be a less effective method of eliminating the toxic effects to plants, especially if the principal effect of plowing were to decrease the concentration of the growth-regulators by bringing up to the surface soil in which it was not present.

The addition of Norit A to soils treated with growth-regulators could eliminate or greatly minimize the toxic effects within a few hours. When the contratotoxicant is mixed in with the sur-

face soil and the soil watered, the adsorption of 2,4-D by the contratotoxicant is apparently rapid, and plantings might be made immediately or soon after such application. Watering of the soil may wash the contratotoxicant into and through the contaminated portions and might enable the growth-regulator to diffuse in the soil and reach the contratotoxicant.

Further studies on the fate of adsorbed 2,4-D are desirable. Much of it is nontoxic to plants as long as it remains adsorbed (14), but its ultimate fate is unknown. Perhaps it could be released later as the charcoal decomposed or with changes of pH value or gaseous content of the soil. Such release could be advantageous or not, depending upon whether one were interested in destroying or in conserving plants grown in such soil. On the other hand, the growth-regulators may be altered chemically in varying degrees.

Norit A, when applied to aerial parts of plants, caused contact damage to the youngest plants, especially Red Kidney beans. Usually the plants recovered by producing new leaves, but they often remained badly damaged. Under higher light intensities these plants would probably have recovered more rapidly and vigorously. With variations of food reserves within the plant, effects could differ greatly. The dark color of the contratoxicants undoubtedly diminished the amount of light entering the leaves, which may have resulted in lessened growth.

After plants were sprayed with BE 2,4-D, it was necessary to apply contratoxicants within about 15 minutes to decrease the toxicity of the regulator. Perhaps the contratoxicants must be applied before the spray dries. It seems more probable, however, that in these experiments a lethal dose of BE 2,4-D might

have entered the plant within 15 minutes following spraying.

The effectiveness of a contratotoxicant applied to plants previously sprayed with a growth-regulator may be partially dependent upon the speed with which the growth-regulator enters the leaf. RICE (10) demonstrated that temperature, light intensity, and the presence of Carbowax 1500 in the solution applied to young Red Kidney bean plants affect the rate of entrance of $\text{NH}_4(2,4\text{-D})$. Under conditions such as that in which $\text{NH}_4(2,4\text{-D})$ in aqueous solution entered the leaf more slowly than BE 2,4-D, contratotoxicants might be effective even after a greater lapse of time following the application of the $\text{NH}_4(2,4\text{-D})$ than of BE 2,4-D.

The base mixture of Weed-No-More when added to suspensions of contratotoxicants decreased their effectiveness. At present it is difficult to explain the chemical and physical factors which produced this effect. Perhaps other types of wetting agents would increase instead of decrease the effectiveness of contratotoxicants. The use of wetting agents might result in even distribution of contratotoxicants over the leaf surface and change the rate of evaporation of droplets.

Summary

1. 2,4-Dichlorophenoxyacetic acid (2,4-D), butyl ester of 2,4-D (BE 2,4-D), copper, 2,4-dichlorophenoxyacetate [$\text{Cu}(2,4\text{-D})_2$], 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and O-isopropyl N-phenylcarbamate (IPPC) were added on June 6, 1946, to separate aliquots of silt loam soil at concentrations of 22 and 220 p.p.m. About two-thirds of each aliquot was placed in storage and occasionally moistened. The remaining one-third of each aliquot was placed in 5-inch

glazed crocks and planted uniformly with Red Kidney beans, white mustard, and barley to test the degree of toxicity of the compounds by later emergence of the plants. Soil was removed from storage on June 27 and November 16, 1946, and May 7, 1947, and similarly planted to test for degree of toxicity of the compounds. The soil planted on May 7, 1947, was replanted on September 2, 1947.

2. In this greenhouse experiment much of the toxicity of IPPC, the least persistent compound used, had disappeared after 12 days of storage. Soil originally containing 220 p.p.m. of 2,4,5-T was still toxic after about 15 months of storage. $\text{Cu}(2,4\text{-D})_2$, 2,4-D, and BE 2,4-D disappeared with about equal rapidity. Soil containing these three compounds at initial concentrations of 22 p.p.m. showed little toxicity after 11 months of storage; soil containing them at 220 p.p.m. had lost its toxicity after 15 months.

3. 2,4-D was added to plots in a lowland soil at a rate of 7.4 lb./acre, and $\text{Cu}(2,4\text{-D})_2$, 2,4,5-T, and BE 2,4-D were added to other plots at chemically equivalent rates. Successive plantings of Sudan grass, Red Kidney bean, white mustard, and soybean were made. Emergence of plants and fresh weight of tops were used as criteria for judging the toxicity in the soil.

4. In this field experiment 2,4,5-T proved to be the least toxic and persistent, and BE 2,4-D the most persistent. The treated soils were still considerably toxic 45 days after application of the growth-regulators.

5. 2,4,5-T brought about poor control of weeds, $\text{Cu}(2,4\text{-D})_2$ and 2,4-D fair control, and BE 2,4-D excellent control.

6. Spading (plowing) resulted in a great decrease in toxicity in a field soil contaminated with 2,4-D. When 10 or 25

lb. of 2,4-D per acre were applied to a field soil, only the upper 3 inches of soil were toxic to plants even after heavy rains.

7. Addition of Zeo-Karb H or Norit A to soil containing 2,4-D resulted in a decrease or in complete elimination of toxicity. In a greenhouse experiment addition of Norit A at a concentration of about 218 p.p.m. decreased the toxic effects of 2,4-D present in the soil.

8. Finely ground Norit A or Zeo-Karb H was dusted or sprayed in aqueous suspension on Red Kidney bean, soybean, white mustard, or marigold. The plants were then sprayed with aqueous 0.1% solutions of ammonium 2,4-dichlorophenoxyacetate, sodium 2,4-dichlorophenoxyacetate, triethanolamine salt of 2,4-D, isopropyl ester of 2,4-D, or BE 2,4-D, or were dusted with a dust containing BE 2,4-D. In most cases the toxic effects of the growth-regulators were greatly decreased or completely eliminated by Norit A or Zeo-Karb H.

9. Super filtrol and attapulugus clays were ineffective as contratotoxicants. Lamp black and bone black were partially effective.

10. The addition of the base mixture of Weed-No-More as a wetting agent to suspensions of contratotoxicants often decreased or eliminated their effectiveness.

11. Red Kidney beans were sprayed with a 0.1% solution of BE 2,4-D and at various intervals after treatment were sprayed with aqueous suspensions of Norit A or Zeo-Karb H. Unless the contratotoxicant was applied within 15 minutes after treatment with the growth-regulator, very little protection was afforded.

12. A 5% aqueous suspension of Norit A heavily sprayed on young Red Kidney beans gave good protection against sprays of growth-regulators. A larger amount of Zeo-Karb H was required for equal protection.

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ABSORPTION AND TRANSLOCATION OF AMMONIUM 2,4-DI- CHLOROPHENOXYACETATE BY BEAN PLANTS¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 597

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Introduction

Several investigators (2, 8, 12) have reported that the addition of Carbowax 1500 to 2,4-dichlorophenoxyacetic acid (2,4-D) markedly increased the responses made to the growth substance when applied to certain plants. With other plants, however, applications of wholly aqueous solutions are just as effective (2). One purpose of the present experiments was to shed some light on the reasons for the increased effectiveness of ammonium 2,4-dichlorophenoxyacetate [$\text{NH}_4(2,4\text{-D})$] when applied to the Red Kidney bean in a solution containing Carbowax 1500. In the present investigation, absorption of $\text{NH}_4(2,4\text{-D})$ by the Red Kidney bean leaf was studied by direct measurement.²

Temperature has been shown (3, 4, 5, 9) to have a marked effect on the herbicidal activity of 2,4-D and related compounds. No detailed studies, however, have been made of the influences of temperature on absorption and translocation of such materials.

Considerable work has been done on the relation of light intensity to absorption and translocation of 2,4-D and related substances (7, 14). It has been reported (7, 14) that local reaction of a treated bean leaf occurs in the dark, the

leaf tip curling back when the growth-regulator was placed near the tip. These investigators were unable to determine how much 2,4-D was absorbed in the dark, because they had no method of measuring absorption directly. Consequently, another objective of the present experiments was to compare the absorption of $\text{NH}_4(2,4\text{-D})$ by the Red Kidney bean leaf under various light intensities.

MITCHELL and BROWN (7) concluded that no translocation of 2,4-D occurred in the dark in the snap bean. They stated, also, that no stem curvature, which was used as a criterion of translocation, resulted during 1 day following application of 2,4-D to snap beans kept in a shaded location (250-500 foot-candles at noon, on a clear day). WEAVER and DEROSE (14) found that kidney-bean plants treated with 2,4-D and kept in the shade did not exhibit as much curvature 1 day after treatment as those treated in the same manner in full sunlight. Despite the close agreement of results of studies on translocation under various light intensities, it seemed desirable to include such a study in the present investigation in conjunction with experiments on absorption.

Material and methods

The Red Kidney bean was chosen as the test plant because its reactions to 2,4-D and related compounds are fairly well known and because of its adaptability to greenhouse conditions and its rapid rate of growth.

All seeds were hand picked to insure

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² The term "absorption" is used in this paper to mean simply the entrance of the ammonium salt into the leaf tissues, irrespective of the forces which are operative or the degree of penetration and later translocation.

uniformity of size and freedom from injury. They were planted in soil in 4-inch glazed pots, three seeds per pot. Before treatment, one plant in each pot was removed, leaving the two most nearly uniform plants. For each of the six experiments 150 pots were planted, but only a maximum of 120 pots with the most nearly uniform plants were used. This procedure insured having all the plants for each experiment very uniform in height and in area of primary leaves. All the plants were grown in the same greenhouse room and were moved to the various test rooms at the desired time before treating with the growth-regulator.

Three test rooms were used for study of the effects of temperature and Carbowax on absorption and translocation. The lowest temperature range (46° – 58° F.) was maintained in a refrigerated room with thermostatic control. The two higher ranges of temperature were maintained within fairly constant limits in rooms in which temperature values did not change rapidly. Basement rooms, all with the same temperature range, were used for the experiments at various light intensities.

Each test room (except the dark room) was equipped with a bank of twelve "daylight" fluorescent tubes which could be raised or lowered so as to regulate the light intensity at the surfaces of the primary leaves to the desired amount. A daily photoperiod of 13 hours was employed while the plants were in the test rooms (except in the dark room).

In all series in the light the plants were treated 2 hours after the lights came on by applying, with a 0.2-ml. Kahn pipette, 0.05 ml. of water containing a known amount of $\text{NH}_4(2,4\text{-D})$ (with or without 0.5% Carbowax 1500 in different series) to the base of the midrib on the upper surface of one primary leaf. The amount

so applied could be controlled consistently within approximately 1 μg . The aqueous solutions of $\text{NH}_4(2,4\text{-D})$ were made by dissolving either 340 mg. or 670 mg. of the salt (the amount depending on whether the treatment was to consist of 34 μg . or 67 μg .) in distilled water and making up to 500 ml. To make the aqueous solution containing 0.5% by weight of Carbowax 1500, 670 mg. of the salt were dissolved in 2.5 gm. of melted Carbowax. After hardening, this was dissolved in distilled water and made up to 500 ml. in a volumetric flask, so that 0.05 ml. would contain 67 μg . of the growth-regulator. One of the primary leaves was treated before much expansion of the first trifoliate leaf had occurred, since preliminary experiments had shown that the responsiveness of kidney-bean plants to a given amount of $\text{NH}_4(2,4\text{-D})$, in terms of both stem curvature and growth inhibition, decreased rapidly with increase in age. Similar results on the effect of age have been reported (16) for several other species.

The plants in the dark room were treated in a similar manner with the aid of a photographic developing lamp with an orange filter. It was found that this light did not allow the formation of chlorophyll in *Avena* coleoptiles in a 24-hour period of exposure. It is probable that such a light had little, if any, effect on metabolic processes in the bean plants during the very few minutes required for application of the solution or during the very few minutes required to clip the treated leaves.

The treated leaves were cut off at the bases of the petioles at various intervals after application (4, 10, 24, 48, and 72 hours), so as to interrupt further translocation of the growth-regulator from the treated leaf to other parts of the plant. Fourteen treated leaves were clipped at

each interval in each series. Nine days after application of $\text{NH}_4(2,4\text{-D})$ to the primary leaves, the fresh weights of the expanded trifoliate leaf blades (including petiolules but not petioles) on treated and control plants were obtained. These weights were used in evaluating the relative amounts of growth inhibition which resulted for the various time intervals during which translocation was possible. The amount of such growth inhibition in similar experiments (14) has been assumed to be proportional to the amount of growth-regulator translocated from the treated leaf into the stem.

If a small amount of a growth-regulator, such as $\text{NH}_4(2,4\text{-D})$, is applied to one side of the stem of a succulent plant, such as bean, cell enlargement is accelerated on the treated side, and stem curvature results (7). Therefore, any stem curvature which follows the application of such a growth-regulator to a leaf blade is an indication that a stimulus has been translocated from the treated leaf to the region of the stem in which the curvature occurs. Some experimenters (2) have used the amount of stem curvature as a measure of the relative effectiveness of various treatments with growth-regulators. In the present experiments the stem curvatures were measured, at the time of clipping the primary leaves, with a transparent plastic protractor.

The clipped primary leaves were placed on moist filter paper in moist chambers and were immediately subjected to the following procedure. Each leaf was placed in a 100-ml. beaker, and the small treated area was washed thoroughly with 20 ml. of distilled water blown forcibly from a pipette. This water was poured into a 25-ml. volumetric flask, and the beaker was rinsed with about 4 ml. of distilled water which was added to the flask. The contents of the

flask were made up to volume with distilled water.

The same general procedure was used in handling the clipped leaves from the untreated control plants. When Carbowax was used in the treatment, an equal amount was also applied in 0.5% aqueous solution to one primary leaf of each of the control plants at the time of treating the other plants in the series.

In the determination of the amount of $\text{NH}_4(2,4\text{-D})$ left on the surface of a treated leaf at the time of clipping, the solution resulting from the washing of the treated area was tested with a Beckman Quartz Spectrophotometer, model DU, according to a slight modification of the method developed by BANDURSKI (1). All density readings were taken at 2300 Å, using 5-cm. silica sample cells. Control blanks, consisting of the washings from control plants, were used in all cases. The density of each blank was checked against that of distilled water. It was found that the density readings of most were similar. However, the density value of an occasional blank would be above or below the usual value, possibly because of some dirt or soluble phenolic compounds washed from the surface of the control leaves. Consequently, it was decided to correct all density readings on the basis of a common blank. The slight variation in the density readings of the blanks indicates that the values of the density readings of the treated-leaf washings may have been affected by the presence of compounds other than $\text{NH}_4(2,4\text{-D})$ in a few instances. However, the actual error, in terms of micrograms of $\text{NH}_4(2,4\text{-D})$ recovered from the leaf, was very small and would tend to average out for any one series in each experiment.

By experimentation the proper combination of treatment, volume of test so-

lution, and sample-cell size was determined, so that most of the density readings on the spectrophotometer would fall within the optimum range of the instrument.

After the last group of treated leaves had been clipped in each series—72 hours after application of $\text{NH}_4(2,4\text{-D})$ —all the plants were removed to a greenhouse room and were allowed to remain until the ninth day after application of the growth-regulator. At this time the fresh weights of the trifoliate leaf blades were obtained. In experiment F these weights were taken on the eleventh day after application.

Results

EXPERIMENT A.—The purpose was to study the effects of temperature on the absorption and translocation of $\text{NH}_4(2,4\text{-D})$ applied in aqueous solution.

The plants were placed in the various test rooms 48 hours before being treated. One room was kept at the temperature range of $46^\circ\text{--}58^\circ\text{F}$. (series 1), one at $79^\circ\text{--}82^\circ\text{F}$. (series 2), and the other at $86^\circ\text{--}92^\circ\text{F}$. (series 3). A primary leaf on each plant was treated, according to the method already described, with $67\text{ }\mu\text{g}$. of the salt. At the time of application the first trifoliate leaf was just starting to expand.

In series 2 and 3 the maximum amount of $\text{NH}_4(2,4\text{-D})$ had entered the leaf within approximately the first 4 hours after application (fig. 1). Maximum absorption in the lowest temperature range (series 1) had occurred at the end of 48 hours or perhaps slightly less.

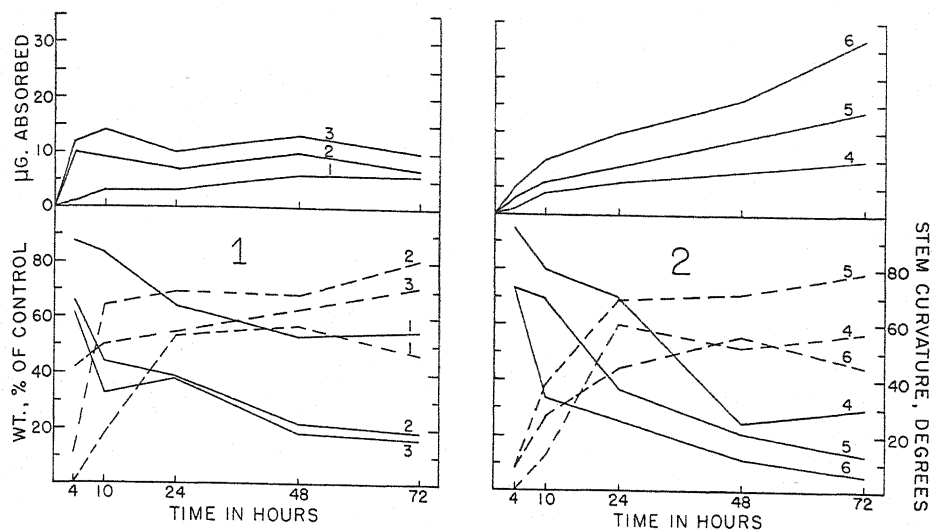
The amount of $\text{NH}_4(2,4\text{-D})$ apparently absorbed was measured as the difference between the known amount applied and the amount washed off the primary leaf. Experimental errors, especially in determining the latter amount, would

help to account for some of the fluctuations in the curves showing apparent absorption in series 2 and 3 (fig. 1). In addition, it will be recalled that each point in a curve is an average of the amounts of $\text{NH}_4(2,4\text{-D})$ apparently absorbed by fourteen plants at the specified time, each group of fourteen consisting of different plants. Moreover, it was found in some cases that individual plants, although outwardly similar to others, showed quite pronounced differences in the amount of the salt apparently absorbed. In spite of these possibilities of experimental error, however, the trends of the curves are definite, and the differences in their values at any one time are for the most part statistically significant (table 1).

The amount of the salt which apparently entered the leaf was greater at the higher temperatures (fig. 1). Table 1 shows the differences between the mean amounts absorbed in the three series for each clipping and, also, for all clippings considered collectively.

Regardless of temperature, the treated primary leaves had to remain attached over 24 hours to allow enough absorption and translocation of the salt to result in approximately maximum inhibition of growth, as measured by the fresh weights of the harvested trifoliate leaf blades (fig. 1).

No stem curvature was found in any plant within 4 hours after application of the salt in series 1, but practically every treated plant in the two higher temperature ranges showed some stem curvature during this period. At the end of this first 4-hour period, the average amount of curvature was greatest at the highest temperature (series 3). However, when the treated leaves were left attached for over 4 hours, the plants in series 2 showed the greatest average stem curva-



FIGS. 1-2.—Effects of temperature and Carbowax 1500 on: (above) absorption of $\text{NH}_4(2,4\text{-D})$ by Red Kidney bean leaves; (below) stem curvature (broken line), and fresh weights of harvested trifoliolate leaf blades of treated plants expressed as a percentage of control averages (solid line). Each plant treated at base of midrib of one primary leaf with $67 \mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in aqueous solution. In series 4, 5, and 6 (fig. 2) solution also contained 0.5% by weight of Carbowax 1500. Abscissa represents time of removal of treated primary leaves after application of growth substance. Light: 900 foot-candles; 13-hour daily photoperiod. Temperature ranges: in series 1, 2, and 3 (fig. 1)— $46^\circ\text{--}58^\circ$, $79^\circ\text{--}82^\circ$, and $86^\circ\text{--}92^\circ$ F., respectively; in series 4, 5, and 6 (fig. 2)— $46^\circ\text{--}58^\circ$, $76^\circ\text{--}80^\circ$, and $86^\circ\text{--}94^\circ$ F.

TABLE 1

EFFECT OF TEMPERATURE ON ABSORPTION OF $\text{NH}_4(2,4\text{-D})$, WITH OR WITHOUT CARBOWAX 1500. DATA BASED ON FOURTEEN PLANTS AT EACH TEMPERATURE RANGE AND EACH CLIPPING. ALL VALUES SHOWN ARE POSITIVE

Series 1, $46^\circ\text{--}58^\circ$ F. Series 4, $46^\circ\text{--}58^\circ$ F.
 Series 2, $79^\circ\text{--}82^\circ$ F. Series 5, $76^\circ\text{--}80^\circ$ F.
 Series 3, $86^\circ\text{--}92^\circ$ F. Series 6, $86^\circ\text{--}94^\circ$ F.

	DIFFERENCE BETWEEN SERIES IN $\mu\text{g.}$ ABSORBED (NEAREST WHOLE VALUE)				
	Time of clipping after treatment				
	4 hours	10 hours	24 hours	48 hours	72 hours
	All clippings				
	$\text{NH}_4(2,4\text{-D})$ in water only				
Series 2—series 1.....	9*	6*	4*	4†	5*
Series 3—series 2.....	2	5	3	3	3*
	$\text{NH}_4(2,4\text{-D})$ in water containing 0.5% Carbowax 1500				
Series 5—series 4.....	2*	2*	3†	6*	9*
Series 6—series 5.....	2	4*	6*	7*	13*

* Significant at 0.01 level.

† Significant at 0.02 or 0.05 level.

ture at any one time through the 72-hour period, despite the fact that the plants in series 3 had apparently absorbed a greater amount of the growth-regulator.

EXPERIMENT B.—This experiment essentially duplicated experiment A except that the aqueous solution of $\text{NH}_4(2,4\text{-D})$ contained 0.5% by weight of Carbowax 1500. A primary leaf of each plant was treated with 67 μg . of the salt. The two higher temperature ranges (series 5 and 6) varied slightly from those in experiment A but were sufficiently like them for a direct comparison of results.

Unlike the results without Carbowax 1500, absorption of $\text{NH}_4(2,4\text{-D})$ continued to take place during the entire 72-hour period in all three series (fig. 2). The total amount absorbed in 72 hours was practically double the amount absorbed at the equivalent temperature range without Carbowax, although absorption during the first 4 hours was slower with Carbowax in the higher temperature ranges.

Again, as with the wholly aqueous treatment, the total amount of the salt absorbed was positively correlated with temperature (fig. 2, table 1).

As in experiment A, the treated primary leaves had to remain attached more than 24 hours in all series to permit sufficient absorption and translocation of the salt to result in approximately maximum inhibition of growth, as measured by the fresh weights of the harvested trifoliate leaf blades (fig. 2).

Likewise as in experiment A, stem curvature did not occur in any plant in the lowest temperature range (series 4) within 4 hours after application (fig. 2). Most treated plants in series 5 and 6 showed some curvature during this period, although the average curvature was not quite so great as in experiment A. The average curvatures were the same in

series 5 and 6 at the end of 4 hours. In all cases in which the treated leaves remained attached to the plants for over 4 hours, the average curvature was greatest in series 5 (intermediate temperature). The average curvature in series 6 was even slightly less, 24 and 72 hours after application, than in series 4 (fig. 2).

EXPERIMENT C.—This experiment was designed to measure the effects of temperature on plants of a slightly younger age to which a smaller amount of $\text{NH}_4(2,4\text{-D})$ was applied. The plants were placed in the various test rooms 48 hours before treating with 34 μg . of the salt in aqueous solution. At the time of application the first trifoliate leaf had not yet started to expand. The temperature ranges in series 7, 8, and 9 were 46°–58° F., 68°–72° F., and 78°–82° F., respectively.

The amount of the salt absorbed was not measured. The fresh weights of the trifoliate leaf blades were inversely correlated with the temperature values maintained during the period of treatment, as in experiments A and B (fig. 3). Again, no stem curvature occurred within 4 hours after application in any plants treated in the lowest temperature range (series 7). In series 8 and 9, however, stem curvature resulted in every plant during this period, and the degree of mean curvature at the end of 4 hours was positively correlated with temperature as in experiments A and B.

EXPERIMENT D.—Half the plants (series 10) were placed in light of 900 foot-candles with a 13-hour daily photoperiod and half (series 11) in darkness, 24 hours before application of $\text{NH}_4(2,4\text{-D})$. The temperature range in both series was 76°–82° F. Each plant was treated with 67 μg . of the salt in water only. At the time of application the first trifoliate leaf had not yet started to expand.

The amount of the salt apparently absorbed in the dark was significantly greater than in the light (fig. 4, table 2). Much of the absorption took place during the first 4-hour period after application, as in experiment A.

There was no stem curvature in the plants treated in the dark, and the average weight of their trifoliolate leaf blades was essentially the same as the average

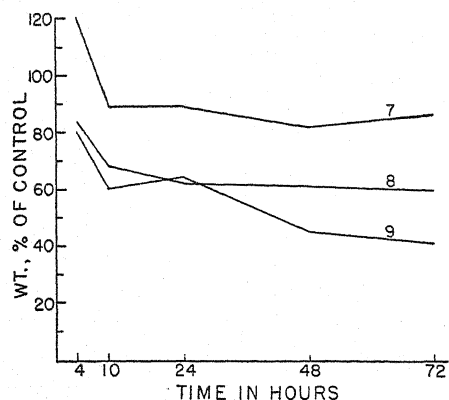


FIG. 3.—Relative growth inhibitions of kidney-bean plants treated, on one primary leaf, with 34 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in aqueous solution, as shown by fresh weights of harvested trifoliolate leaf blades (weights expressed as percentages of control averages). Temperature ranges of series 7, 8, and 9—46°–58°, 68°–72°, and 78°–82° F., respectively. Light: 900 foot-candles; 13-hour daily photoperiod. Abscissa as in figs. 1–2.

of the corresponding controls (fig. 4). The primary leaves treated in the dark folded upward along the midrib and became wavy and crinkled. The average weight of the trifoliolate leaf blades of plants treated in the light was inversely correlated with duration of treatment, as in all other experiments.

EXPERIMENT E.—The plants (series 12) were placed in the dark 48 hours before each was treated with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in water with 0.5% Carbowax 1500 and were left in the dark for the following 72 hours. The temperature of the dark room varied from 82° to

86° F. At the time of application the first trifoliolate leaf was just starting to expand.

There was a continued absorption of the salt in the dark during the entire time that the material remained on the leaf (fig. 4), as was true when Carbowax was

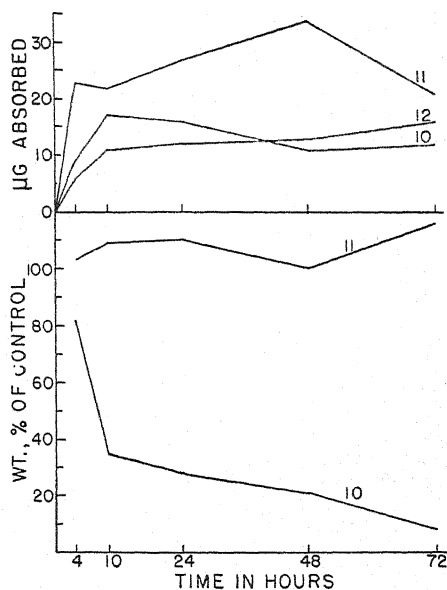


FIG. 4.—Effect of light on: (above) absorption of $\text{NH}_4(2,4\text{-D})$ by bean leaves; (below) stem curvature (broken line) and growth inhibition (solid line), expressed as in figs. 1–2, of plants treated at base of midrib of one primary leaf with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in aqueous solution. Series 12, 0.5% Carbowax 1500 added to solution. Light: series 10, 900 foot-candles with 13-hour daily photoperiod; series 11 and 12, complete darkness. Temperature ranges: series 10 and 11, 76°–82° F.; series 12, 82°–86° F. Abscissa as in figs. 1–2.

used in the treatment in the light in experiment B. There was no stem curvature, but, as in experiment D, the treated primary leaves folded upward along the midrib and became crinkled and wavy. Growth of the trifoliolate leaf blades was not visibly affected by the treatment.

EXPERIMENT F.—Series 13, of eighty plants, was placed in light of 100 foot-candles, series 14 in 900 foot-candles (13-hour photoperiod in both series), and

series 15 in complete darkness, all 24 hours before each plant was treated with 67 μg . of $\text{NH}_4(2,4\text{-D})$ in water only. The temperature range for all series was the same, 69°–77° F. The first trifoliate leaf had not yet started to expand at the time of application.

back at the tips or folded upward along the midrib. They usually became slightly wavy and crinkled also.

The treated primary leaves on plants in light of 100 foot-candles became wavy and crinkled but not to the same degree as those in the dark. There was some

TABLE 2
EFFECT OF LIGHT ON ABSORPTION OF WHOLLY AQUEOUS SOLUTION OF $\text{NH}_4(2,4\text{-D})$
DATA BASED ON FOURTEEN PLANTS FOR EACH SERIES AT EACH CLIPPING

Series 10, 900 foot-candles light Series 13, 100 foot-candles
Series 11, complete darkness Series 14, 900 foot-candles
Series 15, complete darkness

	DIFFERENCE BETWEEN SERIES IN μ G. ABSORBED (NEAREST WHOLE VALUE)					All clippings
	Time of clipping after treatment					
	4 hours	10 hours	24 hours	48 hours	72 hours	
Series 11—series 10.....	Experiment D—Temperature 76°–82° F					
	+14†	+5	+11†	+23*	+9†	+12*
	Experiment F—Temperature 69°–77° F.					
	+1	+1	+2	–1	+1	+1
Series 14—series 13.....	+3	+6*	+4†	+7*	+5*	+5*
Series 15—series 14.....	+4†	+7*	+6*	+6*	+6*	+6*
Series 15—series 13.....						

* Significant at 0.01 level.

† Significant at 0.02 or 0.05 level.

The amounts absorbed at 900 foot-candles and at 100 foot-candles were essentially the same. There were no statistically significant differences between the amounts absorbed by these series at any one time or when all clippings were considered together (table 2).

More of the salt was absorbed in the dark than in either of the series in the light (fig. 5). Most of these differences were statistically significant (table 2).

There was no stem curvature in series 15. The treated primary leaves on these plants in the dark, however, either curled

stem curvature in most of the treated plants in this series, but the average curvature was less at every measurement than for plants at 900 foot-candles (fig. 5). The average curvature in plants at 100 foot-candles was considerably greater 10 hours after treatment than at any other time.

The weight of the harvested trifoliate leaf blades of the plants treated in the dark approximated that of the corresponding controls (fig. 5). This value for plants treated at 100 foot-candles (series 13) was considerably less than the cor-

responding controls in those cases in which the treated leaves remained attached for over 24 hours. The average weight of trifoliate leaf blades (expressed as a percentage of the average of controls) in series 14 was considerably less, however, than in series 13.

In all three series in this experiment, as in all other experiments in which Carbowax was not used, most of the absorption of the salt apparently took place within the first 4-hour period after application.

Discussion

When $\text{NH}_4(2,4\text{-D})$ was applied in water without Carbowax 1500, in most cases the water was observed to evaporate completely, resulting in crystallization of the salt still on the surface, within approximately 4 hours after application in the higher temperature ranges. In these cases it was found that most of the absorption of the salt occurred during this period (figs. 1, 4, 5). In the lowest temperature range the treated area remained moist for a considerably longer period of time, and absorption continued beyond the first 24-hour period after application (fig. 1). These results of measurement of the absorption of the salt in aqueous solution agree fairly closely with the conclusions reached indirectly by WEAVER and DEROSE (14), who, in interpreting the results of WEAVER *et al.* (15), stated that "almost maximum damage ensued if young plants [Red Kidney bean] were not subjected to an artificial rainfall until 6 hours after exposure [to aqueous $\text{NH}_4(2,4\text{-D})$ sprayed on them], indicating maximum entry of the compound into the plant within this period." By "maximum entry" the authors apparently meant the maximum under the conditions of the experiment and not the total amount that could enter the plants under any circumstances.

In all experiments except experiment F, the plants used grew in the greenhouse during warm weather. When they were treated, the drop of solution remained at the base of the midrib of the treated leaf. The plants used in experiment F grew in the greenhouse during cool weather and required several days longer than the others to reach the same apparent stage of development. When their leaves were

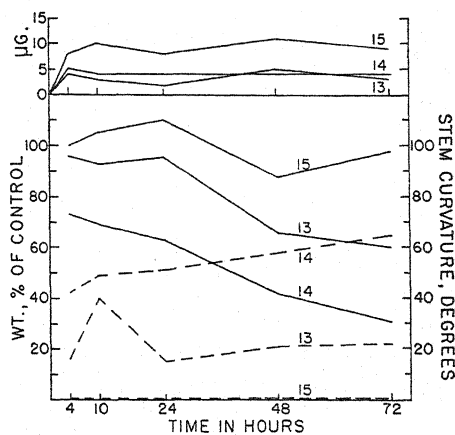


FIG. 5.—As in fig. 4. Plants treated with 67 $\mu\text{g.}$ of $\text{NH}_4(2,4\text{-D})$ in wholly aqueous solution. Temperature range: 69°–77° F. Light intensities for series 13, 14, and 15: 100 foot-candles, 900 foot-candles, and complete darkness, respectively. Daily photoperiod of 13 hours for series 13 and 14.

treated with $\text{NH}_4(2,4\text{-D})$ in water (no Carbowax), the solution tended to creep along the midrib and thence out along the lateral veins. As a consequence, drying occurred quite rapidly, and the amount of the salt absorbed was considerably less than by plants of the same apparent stage of development similarly treated in experiment D. Whether the behavior of the drop was an important factor in the difference in absorption can only be conjectured. SMITH (11), using wholly aqueous solutions of $\text{NH}_4(2,4\text{-D})$ in herbicidal studies, found that sprays of relatively large-droplet size (561–250 μ av. diam.) were more effective than

those of smaller-droplet size ($30\ \mu$ av. diam.). A possible interpretation of his results would suggest that the length of time required for evaporation of the water applied in treatment is an important factor influencing the total amount of the salt absorbed when no hygroscopic agent is added. It should be pointed out that the temperature range in experiment D was somewhat higher than in experiment F. This might account for some of the greater absorption in experiment D. The amount absorbed in experiment F, however, was even less than that absorbed in the lowest temperature range of experiment A by plants in a more advanced stage of development. These observations on the behavior of the drop suggest that there may be differences in the leaf surfaces of plants grown in cool and warm weather which may condition their absorption of aqueous sprays.

In contrast to the limited absorption of the salt after the first 4 hours when applied in wholly aqueous solution are the results obtained when Carbowax 1500 was added to the solution (Figs. 2, 4). At every temperature range tested and in complete darkness as well as in light, the presence of Carbowax 1500 in the solution resulted in the salt being absorbed continuously for 72 hours. A suggestion made by ENNIS and BOYD (2) thus seems to be the correct explanation of the action of Carbowax in increasing the effectiveness of various growth substances applied to the kidney bean. They stated that "the presence of Carbowax effects the retention of moisture on the leaf surface for an interval sufficient to permit absorption of the compound by the epidermal leaf tissues."

It was found that $\text{NH}_4(2,4\text{-D})$ in aqueous solution entered the kidney-bean leaf in the lowest temperature tested ($46^\circ\text{--}58^\circ\text{F.}$), although the rate of entry at low

temperatures was considerably less than at higher temperatures. However, with Carbowax 1500 added, the amount of salt absorbed in 72 hours in the lowest temperature range was as great as in the intermediate temperature range without Carbowax.

In experiments A and B, the total amount of salt absorbed was positively correlated with temperature. It is impossible to say whether the differences in the rates of absorption at various temperatures resulted from the effects of temperature on diffusion phenomena and permeability or whether there were structural differences of the leaves themselves which affected the diffusion of the salt into them. The latter seems doubtful, since the temperature differential was maintained for only 48 hours before application.

No stem curvature in response to treatment was exhibited by any plant in the lowest temperature range ($46^\circ\text{--}58^\circ\text{F.}$) within 4 hours after application, while practically every plant in the intermediate and highest temperature ranges in all experiments showed some curvature within that period. The lack of visible response at the lowest temperature could be a consequence of the effect of temperature on the rates of one or all the following processes: absorption of $\text{NH}_4(2,4\text{-D})$, translocation of the growth-regulator to the stem, or growth responses in the stem. It has been conclusively shown that the rate of absorption was considerably less in the lowest temperature range, so that factor could have been responsible, in part at least, for the lack of stem curvature at the end of 4 hours after application. This does not prove, however, that the other two factors were not important. Another fact which tends to complicate the interpretation of the results of the translocation

studies in low temperatures is that the treated primary leaves had to remain attached for approximately the same length of time in all temperature ranges for maximum growth inhibition (for the given conditions) to ensue. Moreover, no curling or folding of the treated primary leaves occurred in the low temperature range at any time after treatment. This was in marked contrast to the visible responses of primary leaves treated in the dark or in low light intensity and from which complete translocation of the salt apparently did not take place. The last two facts may possibly indicate that most of the growth-regulator which entered the leaf in the low temperature range was translocated into the remainder of the plant just as it was in the higher temperature ranges. However, the evidence is insufficient to determine the effect of temperature on the rate of translocation.

In the present experiments it was found that more $\text{NH}_4(2,4\text{-D})$ actually entered the leaf in the dark than in the light, the differences being highly significant in most cases. Despite this fact, no stem curvature occurred in any plant kept in the dark during the period between application and clipping of the treated leaf. Moreover, the average weight of the harvested trifoliate leaf blades of plants treated in the dark was essentially the same as that of the corresponding controls. These facts suggest either that no translocation of the growth-regulator out of the leaf occurred in the plants kept in the dark or that, even if translocated, it was not effective in evoking responses in the remainder of the plant. Two factors which may have contributed to the greater absorption by the plants in the dark were their greater succulence and the slightly longer time re-

quired for the water applied to them in treatment to evaporate.

Although the amount of the salt absorbed was essentially the same at 100 foot-candles of light as at 900 foot-candles, the average stem curvature was less at every measurement in the former series. The primary leaves treated at the lower intensity tended to become wavy, similar to those in the dark but in lesser degree, while the primary leaves treated at 900 foot-candles were not visibly affected. Moreover, the average weight of the trifoliate leaf blades of plants treated at 100 foot-candles (expressed as a percentage of corresponding controls) was more in every case than those treated at 900 foot-candles. These facts suggest that the limiting factor in the systemic activity of the salt in low light intensities was its slower translocation out of the treated leaf into the stem.

Although relative inhibition of growth of the trifoliate leaf blades was used in the present experiments as a partial indication of the amount of translocation, the writer is fully aware that this is not an absolute measure and used it only because of the lack of a better method. MITCHELL *et al.* (10), after applying 2-iodo-3-nitrobenzoic acid containing radioactive iodine to one primary leaf of young bean plants, found that growth of the terminal buds was reduced roughly in proportion to the amount of the growth-regulator accumulated in them until a concentration of 2.7 $\mu\text{g.}/50 \text{ mg.}$ of dry tissue was reached. At higher concentrations, no greater inhibition occurred. WEAVER (13) reported a strong inverse correlation between the weight of trifoliate leaf blades of the Red Kidney bean and the amount of 2,4-D earlier applied in aqueous sprays. In the present investigation a negative correlation was found between the amount of $\text{NH}_4(2,4\text{-D})$

D) absorbed in experiments A and B and the fresh weight of the harvested trifoliate leaf blades for all plants, regardless of the time of clipping the treated primary leaves. In experiment B this correlation was highly significant (0.01 level) for all plants except those from which the treated leaves were clipped 4 hours after application. In experiment A the correlation was statistically significant for the plants from which the treated primary leaves were clipped after 24 and 48 hours but not for the others. The limited translocation which had occurred during the first 4 hours after application perhaps accounts for this lack of a significant level of correlation in those plants from which the treated primary leaves were then clipped.

There was no consistent statistical correlation between the amount of the salt absorbed and the degree of stem curvature at the time of removing the treated leaf. It was positive, however, for the 4- and 10-hour clippings in experiments A and B and significantly so at both times in experiment B. As previously pointed out, the greatest average curvature after the first 4-hour period consistently occurred in the intermediate temperature range, although more of the salt was absorbed in the highest temperature range. Apparently there are many factors which condition the amount of stem curvature resulting from a given treatment. Consequently, the degree of curvature definitely is not an accurate measure of the amount of a given growth substance which has entered a plant even though such use has been made of it in the past.

In conclusion, Carbowax 1500 apparently increases the effectiveness of various growth substances applied to the kidney-bean leaf in aqueous solution by causing enough moisture to be retained on the treated area to permit absorption

of the material over a longer period of time, thus effecting a greater amount of absorption of the growth-regulator. Carbowax does not appear to have any effect on the rate of translocation of such compounds, although the evidence is insufficient for a definite conclusion. Rate and amount of absorption of $\text{NH}_4(2,4\text{-D})$ by the leaves of kidney-bean plants are positively correlated with temperature. The evidence as to the effects of temperature on its translocation is not conclusive. Rate and amount of absorption of the salt are greater in the dark than in the light, but various light intensities (100 f.-c. or above) do not appear to have any effect on absorption if temperature is constant. There apparently is no translocation of $\text{NH}_4(2,4\text{-D})$ out of the treated leaf in the dark, or, if translocated, it is not effective in evoking responses in the remainder of the plant. The rate of translocation in the light, however, is apparently positively correlated with intensity, at least in the lower intensities. In general, these results substantiate the conclusions of the majority of other workers (7, 14) that translocation of the growth stimulus occurs most rapidly under conditions favoring photosynthesis in, and the movement of food materials from, the treated area.

Summary

1. Young Red Kidney bean plants were treated by applying a known amount of ammonium 2,4-dichlorophenoxyacetate [$\text{NH}_4(2,4\text{-D})$] to the base of the midrib on the upper surface of one primary leaf. The treated leaves were cut off at the bases of the petioles at various intervals (4, 10, 24, 48, and 72 hours) after application so as to interrupt further translocation of the growth-regulator from the leaf to other parts of the plant. Fresh weights of the expanded

trifoliolate leaf blades on treated and control plants 9 days after application of the salt were used in evaluating the relative amounts of growth inhibition which resulted for the various time intervals during which its translocation from the leaf was possible. The average degree of stem curvature at the time of clipping the treated primary leaves was also used as a supplementary indication of the amount of such translocation. To determine how much $\text{NH}_4(2,4\text{-D})$ was apparently absorbed by each leaf, the treated area was washed volumetrically with distilled water. The washings were tested for content of the salt by a slight modification of the method developed by BANDURSKI (1). The amount left on the surface of a treated leaf at any time and thus measured was subtracted from the amount applied to give an approximate measure of the amount absorbed.

2. Much of the absorption of $\text{NH}_4(2,4\text{-D})$ from a wholly aqueous solution by the leaf occurred within the first 4 hours after application. The addition of 0.5% by weight of Carbowax 1500 to the solution resulted in the salt being absorbed continuously during the period of treatment (72 hours).

3. The amount of the salt absorbed was positively correlated with temperature values during treatment, both with and without Carbowax.

4. There was no significant difference between the amounts of the salt absorbed at 100 foot-candles of light and at 900 foot-candles at the same air temperature; the amount absorbed in the dark was significantly greater.

5. The fresh weight of the trifoliolate leaf blades of plants treated in the dark was essentially the same as that of the corresponding controls, and no stem cur-

vature occurred. In addition, the treated primary leaves folded upward along the midrib and became wavy. These facts suggest that, though there may have been movement of $\text{NH}_4(2,4\text{-D})$ within the treated leaf, no translocation from the treated leaf occurred in complete darkness.

6. The relative fresh weights (expressed as a percentage of the average of the controls) of the trifoliolate leaf blades of plants treated at 100 foot-candles of light were considerably greater than those of plants treated at 900 foot-candles, even though the amounts of the growth-regulator absorbed were the same. The average degree of stem curvature was considerably higher in the latter series. Moreover, the primary leaves treated at the lower intensity tended to become wavy, similar to those in the dark but in lesser degree, while those at 900 foot-candles were not visibly affected. All these facts suggest that the rate of translocation of $\text{NH}_4(2,4\text{-D})$ from the leaf in the kidney bean is positively correlated with light intensity prevailing during treatment, at least for the lower intensities.

7. The available data do not indicate any effect of Carbowax 1500 on translocation of $\text{NH}_4(2,4\text{-D})$ from the treated leaf.

8. Data concerning the effects of temperature on the rate of translocation of the growth-regulator were inconclusive.

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INACTIVATION OF 2,4-DICHLOROPHENOXYACETIC ACID IN SOIL AS AFFECTED BY SOIL MOISTURE, TEMPERATURE, THE ADDITION OF MANURE, AND AUTOCLAVING

JAMES W. BROWN¹ AND JOHN W. MITCHELL²

Introduction

Several workers have noted that some time after 2,4-dichlorophenoxyacetic acid (2,4-D) is applied to soils its effectiveness as a herbicide may be markedly reduced or entirely eliminated (1, 2, 3, 5, 6, 7, 8).³ MITCHELL and MARTH (6) found that 2,4-D was still strongly active after stor-

age for 18 months in air-dry soil which was relatively low in organic matter, but in some of their tests it showed no activity in well-manured soil which had been treated with 2,4-D and kept moist for 2 weeks.

The present experiments were under-

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³ Since this paper was submitted for publication the following article was published: KRIES, OLIVE H. Persistence of 2,4-dichlorophenoxyacetic acid in soil in relation to content of water, organic matter, and lime. *BOT. GAZ.* 108:510-525. 1947.

taken to test the effect of levels of soil moisture, soil temperature, the addition of manure, and autoclaving on the inactivation of 2,4-D in soil.

Experimentation

SOIL TEMPERATURE

METHODS.—Experiments were started on September 23, 1946, to evaluate the effect of temperature on the rate of inactivation of 2,4-D in soil. Flats were fitted with wooden partitions so that their 1×2 foot interiors were divided into twelve compartments approximately 4×6×2½ inches. Moisture-proof cellophane liners were constructed and fitted to each of these compartments. To provide drainage and to minimize soil-moisture contamination between compartments, two holes were made in the bottom of each liner directly above apertures in the flats. Fertile, composted soil was placed in each compartment to a depth of approximately 2 inches.

Surface applications of 2,4-D were made at rates of 0.0, 3.5, 7.0, 17.4, 52.2, and 174.0 mg. per pound of soil, approximately equivalent to 0, 2, 4, 10, 30, and 100 lb. per acre. The necessary amounts of 2,4-D for treating all compartments receiving the same rates were dissolved in 60 cc. of 95% ethyl alcohol (warmed gently for the higher rates). The solutions were poured into glass dishes containing enough quartz sand to make a total dry weight of 200 gm. when combined with the weight of the added 2,4-D. The alcohol was evaporated off at 80° C., and the mixtures of 2,4-D and sand were thoroughly stirred in order to insure even distribution of the acid. Aliquots of 5 gm. from each dish thus provided equivalent surface applications of the acid at the desired rates. There were two replications of each treatment in each flat.

All compartments were watered equally. The individual flats were weighed and then stored. During storage, moisture levels were maintained by weight at bi-weekly intervals. The moisture content was approximately 20%, an amount which maintained the soil in a condition suitable for planting. Equal numbers of flats were stored at 36°, 50°, and 70° F., and one flat was removed from each temperature after having been stored for 0, ½, 1, and 2 months.

Upon removal from storage, the flats were transferred to a greenhouse (65°–75° F.). Four rows of seeds were planted in each compartment, alternate rows containing ten mustard and ten barley seeds. Unfortunately, the first two plantings (0 and ½ month) were with freshly harvested mustard seeds which were somewhat dormant, but the last plantings (1 and 2 months) were made with older seed from another source and having a higher percentage of germination.

Emergence counts were made 20 days after planting, and after 35 days fresh weights of plants from the individual rows were obtained.

The sensitivity of mustard to 2,4-D is relatively high, and survival and yield data obtained with mustard were therefore used as a measure of the inactivation of 2,4-D. Barley is relatively resistant to 2,4-D and was used as an indicator of the amount of 2,4-D that could be added to the soil without significantly reducing its growth.

RESULTS.—Survival of mustard increased with storage temperature up to 70° F., the 2,4-D being inactivated at a more rapid rate and to a greater degree at the highest temperature tested (table 1, figs. 1, 2). The activity of 2,4-D applied at a rate of 10 lb. per acre in soil stored for 2 months at 70° F. showed no appreciable effect on the survival of mus-

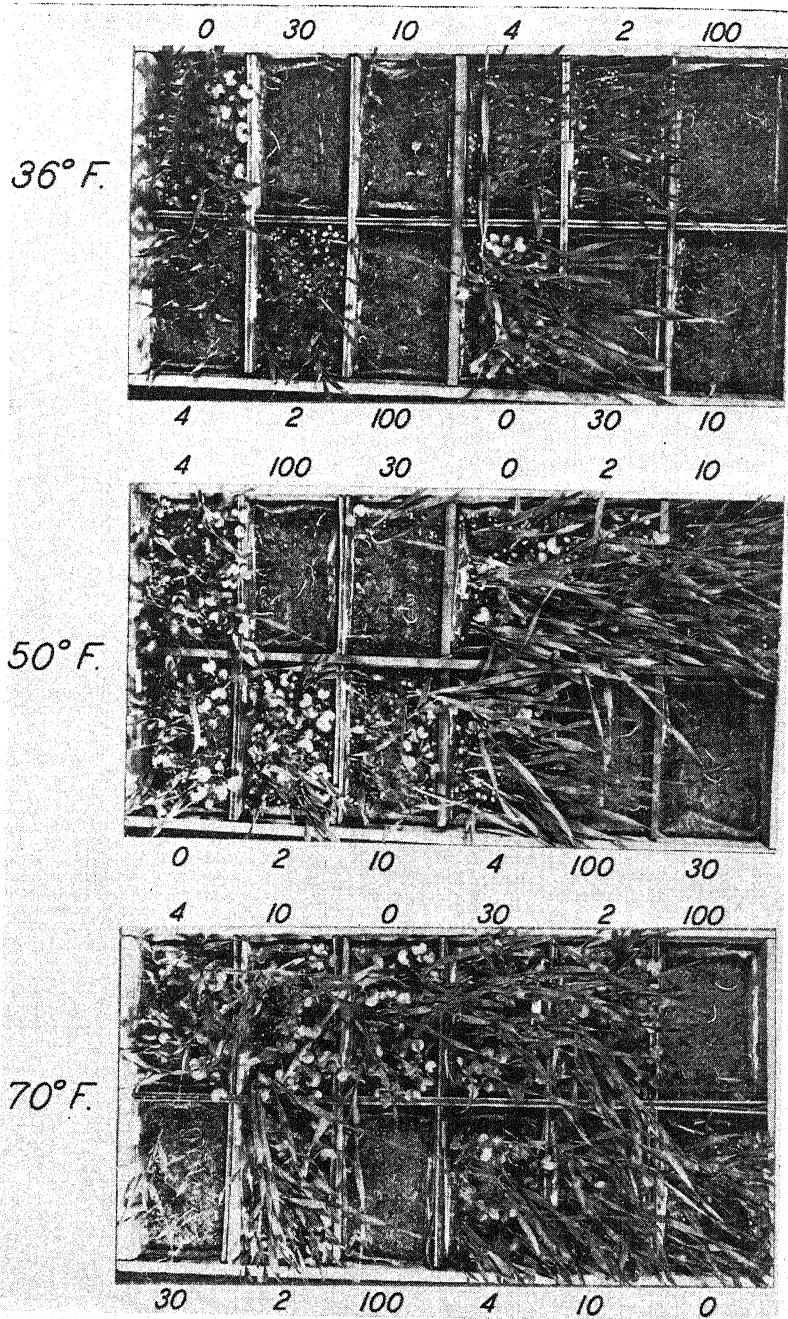


FIG. 1.—Mustard and barley 13 days after planting in soil samples which had been treated with 2,4-D and stored for 2 months at different temperatures. Figures represent equivalent pounds of 2,4-D applied per acre.

tard planted after that time, whereas 2,4-D at a rate of 2 lb. per acre in soil stored at 36° F. for the same length of

sequent growth and development of barley seedlings may be markedly affected (fig. 1). Data on barley yields (table 2)

TABLE 1

SURVIVAL COUNT OF MUSTARD 20 DAYS AFTER PLANTING IN SOIL TREATED WITH VARIOUS AMOUNTS OF 2,4-D AND STORED FOR 0, $\frac{1}{2}$, 1, OR 2 MONTHS AT 36°, 50°, OR 70° F. EACH OBSERVATION REPRESENTS NUMBER OF PLANTS SURVIVING FROM FORTY PLANTED SEEDS

CONCENTRATION OF 2,4-D IN SOIL		MONTHS OF SOIL STORAGE			
Mg./lb.	Lb./acre	0	$\frac{1}{2}$	1	2
Stored at 36° F.					
0.0	0	20	21	31	37
3.5	2	0	4	10	12
7.0	4	0	0	3	0
17.4	10	0	0	0	0
52.2	30	0	0	0	0
174.0	100	0	0	0	0
Stored at 50° F.					
0.0	0	10	25	35	34
3.5	2	0	3	30	33
7.0	4	0	0	32	27
17.4	10	0	0	11	15
52.2	30	0	0	0	0
174.0	100	0	0	0	0
Stored at 70° F.					
0.0	0	13	26	34	35
3.5	2	1	25	37	35
7.0	4	1	15	34	34
17.4	10	0	17	18	32
52.2	30	0	0	3	15
174.0	100	0	0	0	0

Difference required for significance at the 5% and 1% levels = 6.80 and 8.96, respectively.

time was still highly active by the same standards (table 1).

The data obtained on the survival of barley were consistent but are misleading in that emergence of barley is not seriously influenced by the presence of relatively large amounts of 2,4-D, whereas the sub-

TABLE 2

FRESH WEIGHT (GM.) OF TOPS OF BARLEY PLANTS 35 DAYS AFTER PLANTING IN SOIL TREATED WITH VARIOUS AMOUNTS OF 2,4-D AND STORED FOR 0, $\frac{1}{2}$, 1, OR 2 MONTHS AT 36°, 50°, OR 70° F. EACH OBSERVATION OBTAINED FROM 40 PLANTED SEEDS

CONCENTRATION OF 2,4-D IN SOIL		MONTHS OF SOIL STORAGE			
Mg./lb.	Lb./acre	0	$\frac{1}{2}$	1	2
Stored at 36° F.					
0.0	0	54.4	62.6	79.1	87.3
3.5	2	20.9	91.8	90.1	81.4
7.0	4	38.8	64.3	77.7	54.8
17.4	10	8.1	37.9	37.5	37.2
52.2	30	0.8	0.7	2.4	2.4
174.0	100	0.0	0.0	0.0	0.0
Stored at 50° F.					
0.0	0	53.8	70.5	114.7	90.4
3.5	2	36.7	76.8	119.3	84.1
7.0	4	45.2	88.7	125.8	95.6
17.4	10	9.2	37.3	80.4	72.0
52.2	30	0.0	3.5	7.4	13.5
174.0	100	0.0	0.0	0.0	0.0
Stored at 70° F.					
0.0	0	43.5	84.5	129.9	68.0
3.5	2	44.4	69.8	105.5	100.9
7.0	4	24.2	76.4	120.6	75.5
17.4	10	7.7	68.6	90.9	68.6
52.2	30	0.2	3.3	12.3	67.9
174.0	100	0.0	0.8	0.0	4.7

Difference required for significance at the 5% and 1% levels = 24.08 and 31.75, respectively.

indicate that the 2,4-D effect has been minimized after 2 months of storage at 70° F. even at a rate of application of 30 lb. per acre, while storage for the same period at 30° and at 50° F. gave comparable indications for rates of 2 and 10 lb. per acre, respectively.

Since planting dates were staggered because of the different lengths of storage, plants harvested at the end of each test period (35 days) were exposed to seasonal variations of some environmental conditions. Although grown in a greenhouse, growth of the different sets of test plants varied because of these seasonal changes.

SOIL MOISTURE

METHODS.—In studying the inactivation of 2,4-D in soil, duplicate experiments were made to test four soil moisture levels ($2\frac{1}{2}$, 10, 20, and 30%), six rates of 2,4-D application similar to those used in the temperature experiments, two types of application (surface and mixed into the soil), and two storage periods (2 and 4 weeks).

On October 24, 1946, ninety-six aliquots (195 gm. each) of air-dry soil were weighed into moisture-proof cellophane bags shaped to fit 3-inch clay pots. Treatments were then applied to the surface of the soil by sprinkling 5 gm. of quartz sand containing the respective quantities of 2,4-D for the above rates. For the mixed applications, batches of the same air-dry soil were thoroughly mixed with 2,4-D so as to give the same rates of application, calculated on the basis of 2 million lb. as the weight of the surface soil of one acre. Two hundred grams of the mixture of soil and 2,4-D were weighed into each of the cellophane envelopes contained in a second set of ninety-six pots.

The moisture content of the air-dry soil was approximately $2\frac{1}{2}\%$. Pots allotted for each concentration, type of application, and storage period were then brought up to 10, 20, and 30% moisture. Thus, in each group of twenty-four pots, six groups of four pots each contained

different concentrations of the acid, each concentration had four moisture levels, and all twenty-four pots served as one replicate for one type of application. Since each type of application had two replicates, a total of ninety-six pots was included in each storage period employed.

After the moisture levels were adjusted, the cellophane envelopes were closed by sealing them with a hot iron. The initial weights of the pots were obtained, and the pots were stored under brown paper on a bench where the temperature varied from 65° to 75° F.

At the end of 2 weeks, half the pots were removed from storage, the cellophane containers opened, and a hole punched in the bottom of the latter to afford drainage. They were then placed in a shallow tray of water to bring all soil samples to an optimum level for planting seeds. Each pot was then planted with ten selected seeds of Southern Giant Curled mustard (*Brassica japonica*).

The remaining pots were weighed and adjusted to their former weights by the addition of water through a slit cut in the cellophane which was then closed with cellophane tape. The water loss from soil samples (owing to imperfect sealing of the cellophane containers) was less than 5%.

Counts of mustard made 11 days after sowing represented emergence and survival of the seedlings and offered a means of evaluating the inactivation of 2,4-D in the soil.

At the end of 4 weeks the remaining group of soil samples was handled in a similar manner.

RESULTS.—The inactivation of 2,4-D in the soil was influenced significantly by the rate and type of application of the acid, the soil-moisture level during storage, and the length of the storage period

(table 3). There was not a significant difference between replications.

When the 2,4-D was mixed into the soil prior to storage, the survival of mustard planted after the storage period was

TABLE 3

SURVIVAL OF MUSTARD SEEDLINGS 11 DAYS AFTER PLANTING IN SOIL THAT HAD PREVIOUSLY RECEIVED VARIOUS AMOUNTS OF 2,4-D, EITHER APPLIED TO SURFACE OR MIXED INTO SOIL, AND HAD BEEN STORED AT DIFFERENT MOISTURE LEVELS FOR 2 AND 4 WEEKS. FIGURES REPRESENT NUMBER OF SEEDLINGS THAT SURVIVED FROM TWENTY SEEDS PLANTED

CONCENTRATION OF 2,4-D IN SOIL		SURFACE APPLICATION				MIXED WITH SOIL			
Mg./lb.	Lb./acre	Moisture (%)				Moisture (%)			
		2½	10	20	30	2½	10	20	30
		2 weeks' storage				4 weeks' storage			
0.0	0	14	15	15	16	11	17	18	16
3.5	2	0	0	4	16	1	10	14	16
7.0	4	0	0	2	12	0	3	17	15
17.4	10	0	0	0	14	0	0	7	14
52.2	30	0	0	0	1	0	0	0	8
174.0	100	0	0	0	0	0	0	0	0

Difference required for significance at the 5% and 1% levels = 3.8 and 5.0, respectively.

1.4 times greater than when the 2,4-D was applied to the surface of the soil, indicating that the toxic effects of the chemical were reduced as a result of mixing.

A direct relationship existed between the soil-moisture levels of the stored soil and the inactivation of 2,4-D (fig. 3). The

samples containing 2,4-D that had the highest soil moisture during storage later had approximately 122 times as many surviving mustard seedlings as did those with the lowest soil moisture.

Regardless of the conditions used, the toxic effects of 2,4-D decreased as the length of storage increased. The survival of mustard in soil containing 2,4-D that

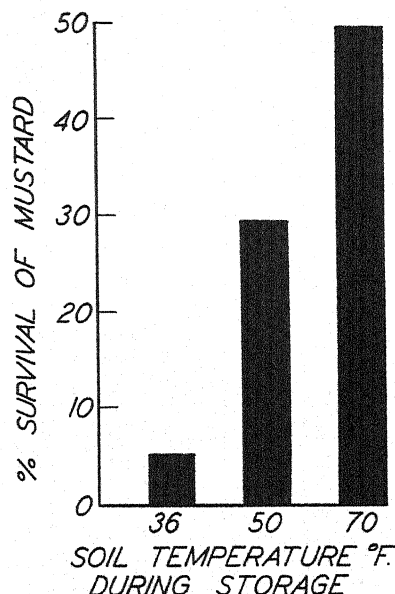


FIG. 2.—Percentage over-all survival of mustard in soil which, prior to planting, was stored at 36°, 50°, or 70° F. Various lots treated with 2,4-D (2, 4, 10, 30, or 100 lb./acre) and stored for 0, ½, 1, or 2 months at each temperature. Survival in untreated soil for each temperature rated as 100%.

had been stored 4 weeks was 2.1 times greater than that of seedlings in comparable samples that had been stored for only 2 weeks.

MANURE

METHODS.—To test the effect of organic matter (cow manure) on the rate of inactivation of 2,4-D in soil, the acid was mixed with soil low in organic matter. Manure was applied to 16-lb. lots of soil without 2,4-D at the rate of 0, 10.8, 21.6,

43.2, and 86.4 gm. per lot and also at the same rates to soil that had received 2,4-D at the rate of 124.8 mg. per lot. These applications were equivalent to manure at the rate of 0, 1000, 2000, 4000, and 8000 lb. per acre, and 2,4-D at the rate of 10 lb. per acre.

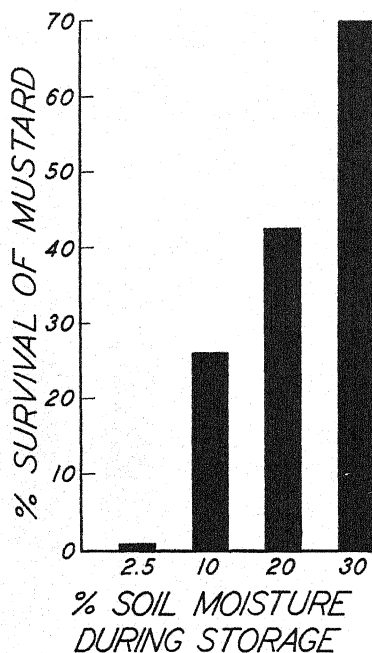


FIG. 3.—Percentage over-all survival of mustard in soil which, prior to planting, was stored with soil moisture content of 2.5, 10, 20, or 30%. Various lots treated with 2,4-D (2, 4, 10, 30, or 100 lb./acre) and held for 2 or 4 weeks with maintained moisture levels. Survival in untreated soil for each moisture level rated as 100%.

Each 16-lb. lot of soil received 400 gm. of quartz sand which was used as a dispersing medium for both the manure and the 2,4-D. The 2,4-D was first carefully mixed with 200-gm. portions of the sand; then this mixture was incorporated into each lot of soil. The manure was first air-dried to obtain a moisture content at which it could be finely ground by means of a hand friction mill. It was pulverized in this way to facilitate mixing it with the

200-gm. portions of sand and ultimately with the soil.

Each treated batch of soil was set up in duplicate so that the amount of inactivation of 2,4-D could be determined at two different time periods. After all samples had been mixed and brought to an optimum moisture content, they were wrapped in brown paper and then sealed in moisture-proof cellophane bags and stored at room temperature. After stor-

TABLE 4

PERCENTAGE EMERGENCE OF MUSTARD PLANTS 8 DAYS AFTER PLANTING IN SOIL CONTAINING 2,4-D AND VARIOUS AMOUNTS OF MANURE COMPARED WITH THAT OF OTHERS PLANTED IN SOIL CONTAINING LIKE AMOUNTS OF MANURE BUT WITHOUT 2,4-D

TREATMENT		STORAGE PERIOD	
2,4-D lb./acre	Manure lb./acre	12 days	21 days
10	0	8.6	0
10	1000	42.0	41.4
10	2000	33.5	30.6
10	4000	23.4	37.4
10	8000	0	0
0	0	78.2	77.5
0	1000	83.0	77.5
0	2000	71.3	84.0
0	4000	93.6	85.5
0	8000	70.0	84.0

age for 12 days, the soil of each treatment was potted in four pots, and mustard seeds were planted to determine the rate of 2,4-D inactivation.

RESULTS.—The rate of inactivation of 2,4-D mixed with soil low in organic matter (without manure added) was relatively slow (table 4, fig. 4). The addition of manure at rates of 1000, 2000, and 4000 lb. per acre greatly increased the rate of inactivation over that of unfertilized soil, but 8000 lb. per acre resulted in a marked decrease in the rate at which 2,4-D was inactivated. This experiment was repeated three times, and results

similar to those presented were obtained on each occasion.

AUTOCLAVED SOIL

METHODS.—An experiment was performed to observe the effect of autoclaving soil containing 2,4-D on the inactivation of this compound during storage.

Wooden partitions were inserted in two greenhouse flats to form in each twelve compartments $6 \times 4 \times 2\frac{1}{2}$ inches. Each compartment was lined with moisture-proof cellophane and then with brown wrapping paper.

Twenty pounds of composted soil were used for each treatment. Treatments

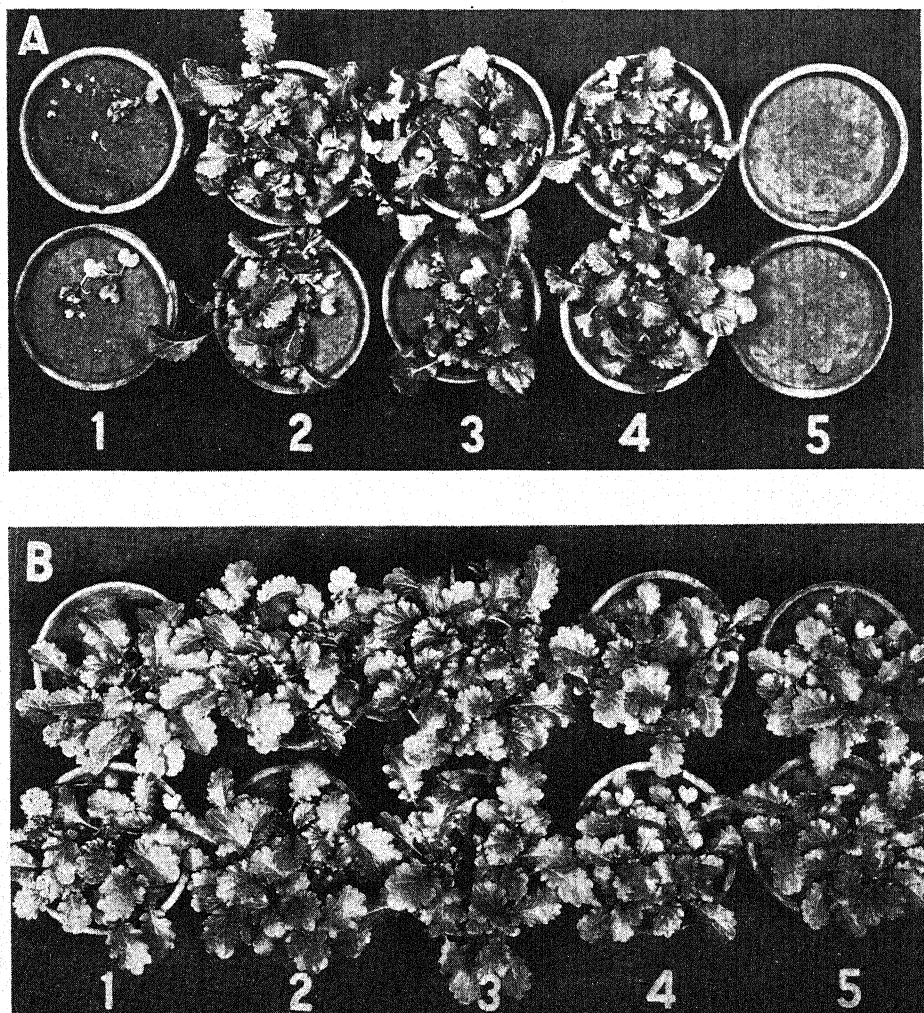


FIG. 4.—*A*, inactivation of 2,4-D (applied at rate equivalent to 10 lb./acre) as indicated by growth of mustard plants in soil containing different amounts of manure: 1, no manure added; 2, 1000 lb./acre; 3, 2000 lb.; 4, 4000 lb.; and 5, 8000 lb./acre. *B*, growth of mustard in soil containing corresponding amounts of manure (rows 1-5) but without 2,4-D added. Plantings made after soil mixtures had been stored for 21 days. Photographed 1 month after planting.

consisted in mixing 2,4-D into the aliquots of soil at rates of 0, 4, and 10 lb. per acre (calculated by surface area of compartments). After thorough mixing, aliquots of 2 lb. of the respective mixtures were weighed into individual compartments so that each treatment was replicated four times in each flat. The flats were then watered until optimum

RESULTS.—The survival of mustard owing to the inactivation of 2,4-D was significantly higher in the soil that had been stored for 2 weeks but not autoclaved (fig. 5). The survival of mustard was not significantly affected by autoclaving untreated soil.

Discussion

Based on these experiments, prolonged toxic effects of 2,4-D in soil would be expected in arid regions or where low soil moisture prevails (5–10% or below). Soil temperatures below about 50° F. apparently retard the rate of 2,4-D inactivation. Aside from soil-moisture effects, it might be expected that 2,4-D would persist for a relatively long period of time under field conditions during cold weather.

The presence of organic matter (manure) in the soil was also a factor that affected the rate of inactivation, but apparently only in extreme cases, such as in the soil that was very low in organic matter and that soil to which relatively large amounts of manure were added. The maximum rate of inactivation of 2,4-D resulted in these experiments when manure was added at the rate of 1000 lb. per acre. The retarding effect on inactivation of the larger amounts of manure (2000–8000 lb. per acre) cannot be explained on the basis of the results obtained so far.

Activity of soil micro-organisms seems to be associated with the process of inactivation of 2,4-D in soil, since its toxic effects remained at a high level during a period of at least 2 weeks in autoclaved soil. In contrast, a large part of the 2,4-D in unautoclaved soil stored under the same conditions of soil moisture and temperature was inactivated during the same period of time. Factors such as soil moisture, temperature, and organic-matter

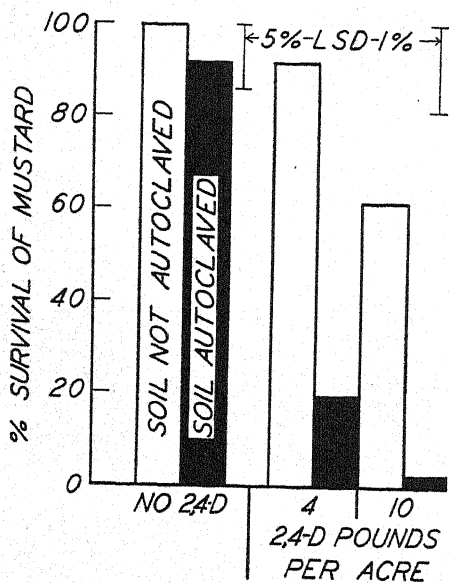


FIG. 5.—Percentage survival of mustard 11 days after planting in autoclaved soil and in soil that was not autoclaved, both of which had been stored for 2 weeks with and without 2,4-D.

moisture for planting was obtained, after which both flats were wrapped in brown wrapping paper. One of the flats was autoclaved for 2½ hours at 114°–116° C. Upon completion of the autoclaving, both flats were wrapped and sealed in cellophane to prevent undue changes in moisture during storage. After storage at 65°–75° F. for 2 weeks, the flats were unwrapped, holes punched in the bottom of each compartment for drainage, and twenty-five mustard seeds were sown in each compartment. Survival counts were made 11 days after the seeds were sown.

content apparently influenced the rate of 2,4-D inactivation indirectly, possibly through their effect on the growth of soil micro-organisms. Some soil microbes are apparently able to decompose 2,4-D when grown in artificial media containing the substance (5).

Other processes, such as leaching (1) and the adsorption or chemical combination of 2,4-D with other substances, no doubt also play a part in the inactivation of 2,4-D in soil. Leaching may result in the movement of 2,4-D throughout the soil so as to decrease the concentration in any one area below the toxic level. LUCAS and HAMNER (4) have shown that 2,4-D is very readily adsorbed by activated charcoal and have suggested that a similar phenomenon may account for the inactivation of 2,4-D in soil. It is assumed, however, that the inactivation of 2,4-D in soil was, in part at least, related to the activity of soil organisms and that factors that favor this activity also favor an increased rate of inactivation of 2,4-D.

It has been noted that 2,4-D is selective in its action regarding certain organisms (10) and that nitrate- and nitrite-forming organisms were definitely injured by 100 p.p.m. but recovered in

10-40 days (9). They were more severely affected by 500 p.p.m., recovery of the nitrite organisms not being observed in 90 days following treatment.

Summary

1. The rate of inactivation of 2,4-dichlorophenoxyacetic acid in soil varied according to the moisture content of the soil during storage; the highest level used (30%) resulted in most rapid inactivation.

2. Inactivation of 2,4-D in soil also increased with the temperature at which the mixtures were stored, the most rapid inactivation occurring at 70° F., the highest temperature used.

3. Applications of 2,4-D that were mixed into the soil were inactivated during a shorter period of time than were like amounts applied to the soil surface.

4. Light applications of manure to soil low in organic matter materially hastened the inactivation of 2,4-D.

5. The inactivation of 2,4-D in soil was significantly reduced by autoclaving.

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WEED CONTROL IN SOILS WITH 2,4-DICHLOROPHENOXYACETIC ACID AND RELATED COMPOUNDS AND THEIR RESIDUAL EFFECTS UNDER VARYING ENVIRONMENTAL CONDITIONS¹

CARL J. C. JORGENSEN AND CHARLES L. HAMNER

Introduction

Previous papers have reported success in destroying weed seed in manure, compost, and soil, using various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and employing different methods of application (1, 2). Although such factors as temperature and moisture have been mentioned (5) as having a bearing on the action and residual effect of 2,4-D, it seemed desirable to attempt more nearly to control these and other factors possibly affecting the weed-seed kill and subsequent retention of 2,4-D and related compounds in soils. Furthermore, previous investigations have covered a rather broad range of concentrations. A narrower range was used in the experiments here described.

Experimentation

APPLICATION OF 2,4-D TO COLOMA SAND

PROCEDURE.—Trials were conducted at Michigan State College, East Lansing, between June and September, 1946. Surface soil was collected from the college orchard and was inoculated with a weed-seed mixture containing more than twenty kinds of weeds, grass, and clover. Among them were such noxious weeds as quackgrass (*Agropyron repens*), yellow foxtail (*Setaria lutescens*), field sorrel (*Rumex crispus*), lamb's-quarters (*Chenopodium album*), bladder campion (*Silene latifolia*), night-flowering catchfly (*S. noctiflora*), yellow rocket (*Barbarea vulgaris*), false flax (*Camelina micro-*

carpa), field peppergrass (*Lepidium campestre*), yellow trefoil (*Trifolium procumbens*), evening primrose (*Oenothera biennis*), heal-all (*Prunella vulgaris*), bracted plantain (*Plantago aristata*), buckhorn plantain (*P. lanceolata*), ox-eye daisy (*Chrysanthemum leucanthemum*), and Canada thistle (*Cirsium arvense*). In addition, the surface soil was later found to contain many additional weed seeds; among them, crabgrass (*Digitaria sanguinalis*), stink grass (*Eragrostis major*), pigweed (*Amaranthus retroflexus*), purslane (*Portulaca oleracea*), mallow (*Malva rotundifolia*), wild carrot (*Daucus carota*), prickly lettuce (*Lactuca scariola*), field sow thistle (*Sonchus arvensis*), and common ragweed (*Ambrosia elatior*).

Three chemicals were used in the experiment. The sodium salt of 2,4-D (sodium 2,4-D) was used at 2, 8, and 16 p.p.m., calculated on the basis of weight of air-dry soil, while the methyl ester of 2,4-D and the acid were used only at 8 p.p.m. Inasmuch as the ester and acid are not soluble in water, they were first dissolved in 95% ethyl alcohol; the solutions were then diluted with water. To incorporate the weed seed and 2,4-D materials thoroughly, the soil containing the weed seed was turned several times while being sprinkled with the aqueous solutions. Fifty cc. of the weed-seed mixture were added to the amount of soil needed to fill sixteen greenhouse flats. The prepared soil was then put into flats and watered sufficiently to moisten thoroughly without leaching.

In order to determine the effect of soil temperature on weed-seed kill and subse-

¹ Journal article no. 872 (n.s.), Michigan State College.

quent residual effect, the flats were placed under four conditions: greenhouse temperature (80° – 105° F.), outdoor temperature (45° – 80° F.), storage temperature (36° F.), and cold-room temperature (0° F.).

The effect of different soil-moisture contents on retention of the toxic effects of sodium 2,4-D was determined by three treatments: (a) saturation, in which the flats were kept very moist at all times; (b) optimum moisture, flats wetted two or three times daily depending upon the weather; and (c) air-dry, with no application of water until planted with test seeds.

To determine the effect of soil reaction on retention of toxic effects of 8 p.p.m. of sodium 2,4-D, two series of soil were treated—one with dilute sulfuric acid and the other with calcium carbonate—to bring the pH values to approximately 4.5 and 7.5, respectively. In all other flats of Coloma sand the pH value was approximately 6.0.

At varying intervals of from 1 to 8 weeks after treatment with growth regulators, designated flats were planted so that each contained ten seeds of Golden Bantam sweet corn, ten seeds of Little Marvel garden pea, and twenty seeds of Scarlet Globe radish. These plants were chosen since they represent the grass, legume, and crucifer families, respectively, and, in addition, are relatively rapid in germination. Each flat was divided into four sections crosswise. At the end of the desired time interval, the section of soil to be planted was loosened, furrowed, planted, and carefully raked. The remaining sections of the flat were not disturbed, in order to permit any weed seedlings to develop. Before planting, flats stored at cold temperatures were set out on the greenhouse benches for 24 hours in order to bring their temperature

to that of the greenhouse. They were left in the greenhouse after planting. The outdoor flats were left outside throughout the experiments.

The residual effects of sodium 2,4-D in the soil were also observed on tomato-seedling transplants which were planted in various flats 4 weeks after treatment with the salt.

VARIOUS 2,4-D MATERIALS.—During the 8 weeks of the experiment weed seeds germinated, and the weeds grew rapidly in untreated flats in the greenhouse, while none appeared in any of the treated flats under similar conditions. Corn, peas, and radishes planted in the untreated soil all grew vigorously except the first planting of radish, in which seed of low germination percentage was used. Lots with high germination percentages were used in subsequent plantings.

Corn planted 1 week after treatment of the soil with the methyl ester of 2,4-D appeared unaffected by the growth regulator (table 1). That planted 1 week after soil treatment with 2,4-D appeared slightly affected, and that planted 1 week after soil treatment with sodium 2,4-D was affected considerably. Most frequently the affected sprouts were etiolated and showed definite curvature. Later, the leaves showed various degrees of curling and twisting. Some curled inward toward the base of blade; others showed a yellowing of the tips. Many corn seedlings failed to appear above the soil surface and, when dug up, had bent so severely that the tips were growing downward. Dwarfing or no growth was found in the case of peas and radish planted 1 week after soil treatment.

Corn planted 3 weeks after treatment of soil grew normally in all flats. Peas planted 3 weeks after treatment of soil with either the ester or the acid germinated poorly, while those planted in

soil containing the sodium salt did not germinate. Similarly, radish failed to germinate in both salt- and acid-treated soils and germinated poorly in the ester-treated flats.

In plantings 5 and 7 weeks after soil treatment, pea, corn, and radish all grew satisfactorily with little or no difference among treatments, thus indicating that

taining the methyl ester of 2,4-D under optimum moisture conditions.

EFFECTS OF TEMPERATURE.—Differences in growth of test seedlings under greenhouse and outdoor temperatures (table 2) did not result solely from the effect of temperature on the activity of sodium 2,4-D during the growth period or during the pre-planting period. The

TABLE 1
GROWTH OF TEST SEEDLINGS UNDER OPTIMUM MOISTURE AND GREENHOUSE TEMPERATURES IN SOIL TREATED WITH 2,4-D MATERIALS
(+++ , excellent growth; ++ , fair growth; + , poor growth; o , no growth)

TIME INTERVAL BETWEEN TREATMENT AND PLANTING (WEEKS)	CONCENTRATION USED (P.P.M.)	METHYL ESTER OF 2,4-D			SODIUM 2,4-D			2,4-D		
		Corn	Pea	Radish	Corn	Pea	Radish	Corn	Pea	Radish
1.....	{ 0 8	+++ +++	+++ o	++ o	+++ +	+++ o	++ o	+++ ++	+++ +	++ o
3.....	{ 0 8	+++ +++	+++ +	+++ +	+++ +++	+++ o	+++ o	+++ +++	+++ +	+++ o
5.....	{ 0 8	+++ +++	+++ +++	+++ +++	+++ +++	+++ ++	+++ +++	+++ +++	+++ +++	+++ +++
7.....	{ 0 8	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++	+++ +++

the toxic effect of 2,4-D had been removed from the soil in some way.

From these results (table 1) it would appear that, under conditions of optimum moisture and greenhouse temperature, all three materials are equally effective as weed-seed killers in the soil, while the ester has an added advantage in that its toxic effects disappear from the soil more rapidly; soil treated with it can thus be planted sooner after treatment. Although no attempt was made to test the ester or acid under conditions of soil saturation, saturated flats containing sodium 2,4-D showed a more rapid disappearance of toxicity than flats con-

authors are of the opinion that the better performance of corn in the greenhouse in the 2- and 3-week plantings may be the result of the more nearly optimum temperature range for germination of corn in the greenhouse. Conversely, the better performance of peas and radish under outdoor temperatures may reflect better growing conditions for these cool-season crops. It was also noted that the flats outdoors dried less rapidly and were probably maintained at a higher moisture percentage than those in the greenhouse. The residual toxicity, as shown by the test species, under both greenhouse and outdoor temperatures disappeared in 5

weeks for the 16-p.p.m. concentration and in 3 weeks for the 2-p.p.m. concentration of sodium 2,4-D.

It was thought best to spread the planting tests on flats stored at 36° F. and 0° F. over a longer period, inasmuch

poorer growth of peas, corn, and radish resulted than in flats held at greenhouse temperature for 1 week before seeding, while flats kept at 0° F. for 4 weeks and then brought into the greenhouse and seeded showed growth of test plants

TABLE 2

GROWTH OF TEST SEEDLINGS UNDER OPTIMUM MOISTURE AND GREENHOUSE AND OUTDOOR TEMPERATURES AFTER SOIL HAD BEEN TREATED WITH SODIUM 2,4-D AND STORED AT OPTIMUM MOISTURE AND INDICATED TEMPERATURES FOR VARIOUS INTERVALS
(Symbols as in table 1)

TIME INTER- VAL BE- TWEEN TREAT- MENT AND PLANT- ING (WEEKS)	TREAT- MENT (P.P.M.)	GREENHOUSE TEMPERATURE (80°-105° F.)			OUTDOOR TEMPERATURE (45°-80° F.)			STORAGE TEMPERATURE					
		Corn	Pea	Radish	Corn	Pea	Radish	(36° F.)			(0° F.)		
								Corn	Pea	Radish	Corn	Pea	Radish
1.....	0	+++	+++	++
	2	+++	++	++
	8	++	+	+
	16	+	o	o
2.....	0	+++	++	+++	++	+++	+++
	2	+++	++	+++	++	+++	+++
	8	++	+	+	++	+	+
	16	++	o	o	++	+	+
3.....	0	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++
	8	+++	++	++	+++	++	++	+++	++	++
	16	+++	o	o	+++	+	+	+++	o	o
4.....	0	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++
	8	+++	++	++	+++	++	++	++	o	o
	16	+++	o	+	+++	+	+	++	o	o
5 or 6...	0	+++	+++	+++	+++	+++	+++	+++*	+++*	+++*
	2	+++	+++	+++	+++	+++	+++	+++*	+++*	+++*
	8	+++	+++	+++	+++	+++	+++	+++*	+++*	+++*
	16	+++	+++	+++	+++	+++	+++	+++*	+++*	+++*
8 or 9...	0	+++†	+++†	+++†	+++†	+++†	+++†
	2	+++†	+++†	+++†	+++†	+++†	+++†
	8	+++†	+++†	+++†	+++†	+++†	+++†
	16	+++†	+++†	+++†	+++†	+++†	+++†

* 6 weeks.

† 8 weeks.

‡ 9 weeks.

as in previous experiments a more rapid disappearance of toxicity was noted under higher temperatures. The results obtained (table 2) confirmed these observations, with some exceptions. The relatively long retention of toxicity from sodium 2,4-D in the soil under freezing and near-freezing temperature is definitely indicated. When treated flats, kept at 36° F. for 3 weeks, were brought into the greenhouse and immediately seeded,

similar to that in flats stored for 1 week at greenhouse temperatures. Further planting tests showed no observable difference in the degree of toxicity between flats kept for 4 or 9 weeks at 0° F. This would indicate a retention of sodium 2,4-D in the soil for long periods of time under subfreezing temperatures.

Treated flats containing sodium 2,4-D, kept at 36° F. for several weeks, showed a very slow disappearance of toxicity.

Treated flats held for 8 weeks in storage at 36° F. and then placed on greenhouse benches and planted with peas, corn, and radishes showed that toxicity, although still present in the soil, was not so great as in soil held for 3 weeks at 36° F. This was indicated by better growth of the corn and radishes in the 8-week flats.

mitted satisfactory germination and growth of the test plants by the end of 3 weeks at all pH levels. At 16 p.p.m. the toxicity disappeared within 7 weeks.

MOISTURE VARIATION.—In flats containing sodium 2,4-D in saturated soil, the toxicity to test plants disappeared much sooner than in the flats kept at

TABLE 3
GROWTH OF TEST SEEDLINGS UNDER OPTIMUM MOISTURE AND GREENHOUSE TEMPERATURES
IN SOILS OF DIFFERENT PH VALUE TREATED WITH SODIUM 2,4-D AS INDICATED
(Symbols as in table 1)

TIME IN- TERVAL BE- TWEEN TREATMENT AND PLANTING (WEEKS)	TREAT- MENT (P.P.M.)	Ph 4.5			Ph 6.0			Ph 7.5		
		Corn	Pea	Radish	Corn	Pea	Radish	Corn	Pea	Radish
1.....	0	+++	+++	++	+++	+++	++	+++	+++	++
	2	++	+	+	++	+	+	++	+	+
	8	+		o	+		o	+		o
	16	+	o	o	+	o	o	+	o	o
3.....	0	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++
	8	+++	o	o	+++	o	o	+++	o	+++
	16	+++	o	o	+++	o	o	+++	o	+
5.....	0	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++
	8	+++	++	+++	+++	+++	+++	+++	+++	+++
	16	+++	++	++	+++	+++	+++	+++	+++	+++
7.....	0	+++	+++	+++	+++	+++	+++	+++	+++	+++
	2	+++	+++	+++	+++	+++	+++	+++	+++	+++
	8	+++	+++	+++	+++	+++	+++	+++	+++	+++
	16	+++	+++	+++	+++	+++	+++	+++	+++	+++

Peas in the latter planting, however, failed to germinate.

EFFECTS OF SOIL REACTION.—Results of this experiment were rather striking. The reaction of the soil had little influence on the loss of toxicity in soil treated with sodium 2,4-D (table 3). The minor growth differences in the soils at the three pH levels indicate that the toxic effects of sodium 2,4-D disappear slightly faster in near neutral or alkaline soils. A treatment of the soil at 2 p.p.m. per-

optimum moisture, while in those flats stored under air-dry conditions, very little toxicity was lost, as indicated by the subsequent poor growth of pea and radish (table 4). Flats containing sodium 2,4-D in the soil under air-dry conditions showed little loss of toxicity during 8 weeks. Plantings after 8 weeks of storage were as much inhibited as those after 4 weeks. Germination of test seeds was unimpaired in flats which had been stored for 3 weeks at saturation level,



FIGS. 1-3.—Relative growth of corn, peas, and radish in Coloma sand treated with sodium 2,4-D at rates of (left to right) 0, 2, 8, and 16 p.p.m. Fig. 1, soil saturated at all times; nos. 1, 2, and 3 indicate plantings 1, 2, and 3 weeks after soil treatment. Fig. 2, soil kept under optimum moisture conditions; nos. 1, 2, 3, and 4 indicate plantings 1, 2, 3, and 4 weeks after soil treatment. Fig. 3, soil stored air-dry for 2 weeks after treatment and then watered and planted.

grass, ragweed, lamb's-quarters, plantain, yellow rocket, and crabgrass. Considerable red, alsike, and white clover, alfalfa, and sweet clover also germinated.

In the flats treated with 2 p.p.m. of sodium 2,4-D, an estimated 2% of weed seed germinated and grew; the flats containing 8 and 16 p.p.m. of sodium 2,4-D were entirely weed-free. Among those weeds observed to resist the 2-p.p.m. treatment were stink grass, red and alsike clover, dock, pigweed, lamb's-

tomato transplants are considerably more resistant to sodium 2,4-D than are the other young weed and crop seedlings in the tests.

Although 8 and 16 p.p.m. of sodium 2,4-D in soil effectively prevented germination and growth of weeds and test plants under many conditions, the authors observed mushrooms growing normally in otherwise plant-free flats. These were principally the cup (*Sarcoscypha*) and gill-cap (*Marasmius*) types.

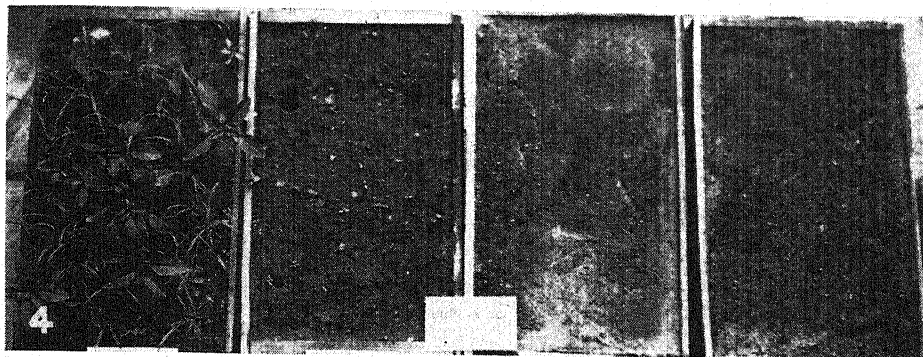


FIG. 4.—Relative growth of weeds in Coloma sand 4 weeks after soil was treated with sodium 2,4-D at rates of (left to right) 0, 2, 8, and 16 p.p.m.

quarters, yellow rocket, campion, and plantain. Figure 4 shows the relative growth of weeds 4 weeks after soil treatment in flats left in the greenhouse. Similar results of soil treatment were noted when flats had been placed under outdoor temperature conditions.

MISCELLANEOUS OBSERVATIONS.—Tomato seedlings transplanted 4 weeks after soil treatment into flats containing sodium 2,4-D at 2, 8, and 16 p.p.m., grew normally in flats stored under normal greenhouse and outdoor conditions. When planted in treated flats which had been kept air-dry or at low temperatures and then brought to greenhouse conditions, growth was inhibited or the plants showed stem curvature and, in most cases, death. It would appear that young

APPLICATION OF SODIUM 2,4-D TO MUCK

Trials on muck were conducted in a lesser way. Surface muck was collected from the Michigan State College muck-trial plots, inoculated with the weed-seed mixtures used in the previous experiment, and treated with sodium 2,4-D at a concentration of 8 p.p.m. only. Temperature and moisture were the only two variables tested. In addition to the weed seed added, the muck was later found to contain many weeds—among them crabgrass, nut grass (*Cyperus esculentus*), wormseed mustard (*Erysimum cheiranthoides*), lady's-thumb (*Polygonum persicaria*), witchgrass (*Panicum capillare*), sow thistle, rough cinquefoil (*Potentilla norvegica*), and hound's-tongue (*Echium vulgare*). The flats containing muck were

placed on the benches along with the sand flats and given the same general treatment.

Although 2 p.p.m. of sodium 2,4-D were very effective in destroying weed seeds in Coloma sand, 8 p.p.m. applied to muck resulted in little, if any, inhibition of weed growth. Furthermore, plantings of corn, peas, and radish in treated muck under all variations of temperature and moisture germinated well, and the seedlings appeared normal. One exception was a slight curvature of corn sprouts in flats kept at 36° F. and 0° F., for 3 and 4 weeks, respectively, and then planted. This effect disappeared rapidly, and the plants then grew normally.

The muck controls predominated in such weeds as nut grass, wormseed mustard, sow thistle, lady's-thumb, crabgrass, witchgrass, rough cinquefoil, and hound's-tongue. The treated flats contained the same quantity and distribution of weeds as the controls, attesting to the lack of inhibitory effect of the chemical. Both controls and treated flats contained numerous clover plants of several kinds.

It has been reported by LUCAS and HAMNER (4) that activated charcoal may inactivate 2,4-D. Certain muck soils, under certain conditions, may also apparently inactivate sodium 2,4-D.

Discussion

Of the three 2,4-D materials tested, no great differences in the degree or persistence of their toxicity could be observed in soils kept at optimum moisture and greenhouse temperatures. The methyl ester of 2,4-D permitted subsequent plantings a little sooner than either the acid or the sodium salt. This probably resulted from the volatilization of the

ester under the high greenhouse temperatures. Under watering conditions permitting leaching, the acid and salt would no doubt disappear sooner also. The three compounds were equally effective weed-seed killers at 8 p.p.m. in sand and permitted normal subsequent plantings within 5 weeks.

Under all treatments and concentrations, corn was more able to resist the toxic effects of 2,4-D materials than were pea or radish. This substantiates previous statements that grasses as a group are more resistant than dicotyledons, both to selective herbicidal 2,4-D sprays above ground and to treatments incorporating such chemicals in the soil. The rapid germination and sensitivity of radish seed would indicate the feasibility of using it as an indicator plant under greenhouse or outdoor conditions.

The storage of treated flats at temperatures of 36° F. and 0° F. resulted in retention of toxicity of sodium 2,4-D for long periods of time as compared with flats subjected to normal outdoor and greenhouse temperatures during summer.

Toxicity from sodium 2,4-D was dissipated rapidly under high soil moisture conditions and in air temperatures ranging from 50° to 80° F. This was probably a twofold action. Leaching action undoubtedly removed some of the chemical (3). Also, under these high moisture and temperature conditions, the microbial population increased rapidly, and it is possible that the 2,4-D compounds were broken down by soil microorganisms. The fact that air-dry flats retained their toxicity for a long time would indicate the need for caution in using these materials during periods of drought. Should the methyl ester of 2,4-D be used under such conditions, it is conceivable

that it would volatilize at high temperatures without effecting a weed-seed kill. Indications are that, with any 2,4-D material used, a high moisture content of the soil should follow its application, either by natural or artificial means, in order to permit early use of the soil.

The results obtained with 8 p.p.m. of sodium 2,4-D on muck are somewhat inconsistent with earlier findings (2), since, in this experiment, weeds were not controlled. It is generally believed that substances adhering to colloidal surfaces are unavailable to plants under many conditions. Also, populations of micro-organisms are much greater in organic soils, and, if these use 2,4-D for food, it would naturally disappear more rapidly. Further experimentation may show the correlation between percentage of organic matter and inhibition of weed and crop growth in soils treated with 2,4-D materials.

Finally, the experiment would point to the following practical applications. (a) Soils of a sandy nature can be made weed-free for subsequent plantings by the addition of low concentrations of 2,4-D materials under outdoor or greenhouse conditions. (b) Because of the low concentrations necessary, the cost of materials for gardens and acreages would not be prohibitive; 2 p.p.m. would correspond to 2 pounds per acre to a depth of 3 inches. (c) The materials can be readily put into solution and evenly distributed.

Summary

1. In Coloma sand an application of 2 p.p.m. of the sodium salt of 2,4-dichlorophenoxyacetic acid (sodium 2,4-D) killed from 95% to 98% of all weed seed present in the soil, while 8 or 16 p.p.m. made the soil virtually weed-free. Seeds of weedy grasses were killed in sand with concentrations of 8 and 16 p.p.m. of sodium 2,4-D.

2. There were no appreciable differences in weed-seed kill by the acid, the sodium salt, or the methyl ester of 2,4-D applied to Coloma sand.

3. Higher temperatures resulted in disappearance of toxicity of sodium 2,4-D from the soil more rapidly than did freezing or subfreezing temperatures, while the compound was equally effective as a weed-seed killer under all these temperatures.

4. Differences in pH value of Coloma sand did not appreciably affect the rate of loss of toxicity under the conditions of the experiment.

5. In water-saturated flats, sodium 2,4-D apparently disappeared in 3 weeks, while in air-dry flats toxicity was still present after 8 weeks.

6. Eight p.p.m. of sodium 2,4-D were ineffective in killing weed seeds in muck.

7. Corn was more highly resistant to 2,4-D materials under all conditions than either pea or radish.

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USE OF 2,4-DICHLOROPHENOXYACETIC ACID HERBICIDES ON SOME WOODY TROPICAL PLANTS¹

KENNETH V. THIMANN

Introduction

A large number of recent papers have described the use of substances of the auxin type to kill herbaceous weeds such as dandelion, mustard, water hyacinth, etc. (1, 3, 4, 7, 9), but few have dealt with woody plants. Many important weeds are woody perennials. This is particularly true of southern and tropical climates. The rapid growth rate of plants in the tropics makes successful weed control of the greatest importance there (10, 11).

In Cuba the most serious weed is "Aroma marabu" (*Dichrostachys nutans* Benth.), a woody leguminous perennial (introduced from Africa some 70 years ago) which grows into almost impenetrable thickets about 12 feet high, extremely thorny and resistant. When cut down, the plants grow vigorously from the stumps and soon re-establish themselves; it is common practice to cut the plants back to the stumps two or even three times a year. The writer has seen plots in which the plants have been cut back in this way three times a year for 17 years; they still grow vigorously. The root systems are extensive and give rise to shoots at considerable distances from the parent plant. It was thought worth while, therefore, to investigate the susceptibility of these plants to 2,4-dichloro-

phenoxyacetic acid (2,4-D). At the same time some observations were made on "Guao" (*Comocladia dentata* Jacq.), a member of the Anacardiaceae whose leaves cause a skin irritation like that caused by poison ivy, and which is also difficult to eradicate by cutting.

It is hard to assess the killing of a woody plant. Curling of young tips is, of course, only evidence of auxin entry; even defoliation is evidence only of toxicity and not of death. Failure of new buds to develop from the root crown within a reasonable period is *prima facie* evidence of death, but even this cannot be relied on, particularly in plants with great powers of regeneration. What follows is, therefore, in the nature of a preliminary report.

Material and methods

The following points seemed important to investigate: the concentration required, the relative effectiveness of different preparations, the influence of time of day, sun and shade, and the possibility of large-scale application.

For the sodium salt of 2,4-D, "2,4-Dow," containing 70% of the acid in salt form, was used in aqueous solution; for the ester, "Weedone," containing 9.6% of the acid in ester form, was used.² In one instance the free acid (tech. grade) was used in aqueous solution with 0.5% Carbowax 1500. Spraying was done with an ordinary 3-gallon knapsack sprayer and later with a 50-gallon track-mounted

¹ Publication no. 1, journal series, from the Atkins Garden and Research Laboratory of Harvard University, Soledad, Cienfuegos, Cuba, at which the experiments were carried out. The author's thanks are due to Dr. ARTHUR G. KEVORKIAN, Director, for his helpful co-operation and assistance and for examining some of the treated plants after the author's departure.

² The author wishes to thank the Dow Chemical Company and the American Chemical Paint Company for generous samples of their products.

container and a powerful hydraulic pump.³

The plants, both Aroma and Guao, consisted of vigorous 1 year's growth from old stumps; they were 1-2 feet high and 2-4 feet in diameter. Although it was the dry season (February and March), all were growing reasonably well.

Since it would be impractical to carry out experiments in sequence if one had to wait for evidence of killing, some preliminary criterion of toxicity was necessary. Leaf discoloration was used for this. Since each plant consists of a number of long stems, each with up to twenty-five leaves, the leaves on each stem were checked for (a) clear-cut yellowing and (b) obvious death (brown and dry). The percentage of leaves in these two conditions was determined for each stem, and then the values for the stems were averaged to give a final number for the plant as a whole. Counts of this sort were made at periods up to 3 weeks after spraying. Thereafter plants were classified as: (1) apparently killed; (2) some green leaves but no growing points present, i.e., doubtful; (3) growing points present or probably so; and (4) plants essentially intact or evidently alive. With the Guao, groups (2) and (3) could be substituted by (2) seriously, and (3) slightly damaged, respectively.

Experimentation

AROMA

INFLUENCE OF CONCENTRATION.—It was clear from the literature that the concentrations needed would be above 1 gm./l. In the first experiments the effectiveness of a concentration of 2 gm./l. was compared with that of 3 gm./l. This was done with the sodium salt (2,4-Dow)

³ Contributed by the Compañía Azucarera de Soledad, to whose staff the author's thanks are due for their most helpful co-operation.

which, being 70% 2,4-D acid, was used at 2.8 and 4.3 gm./l., respectively. Two gallons were used for about 400 square feet. Results are given in table 1. The greater toxicity of the higher concentration is quite clear.

INFLUENCE OF DOUBLE SPRAYING.—The same patch was divided in half, and 5 days after the first spraying one half was sprayed a second time, using 1 gallon for about 200 sq. ft. Table 1 shows that

TABLE 1
INFLUENCE OF CONCENTRATION AND
NUMBER OF SPRAYINGS

	7 DAYS AFTER SPRAYING		12 DAYS AFTER FIRST SPRAYING		
	2 gm./l.	3 gm./l.	2 gm./l.	3 gm./l.	Sprayed twice * 2 gm./l.
% of leaves yellowed...	34	65	72	94	94
% of leaves killed.....	3	5	32	77	74
No. of whole plants counted....	25	16	7	17	9

* Second spraying 5 days after first.

the second spraying greatly increased the effectiveness but that the results were almost exactly the same as with one spray at 3 gm./l. A single spraying at 3 gm./l. was therefore decided on as the basic treatment.

INFLUENCE OF SUN AND SHADE.—To determine whether the rapid discoloration of leaves was a function of exposure, an area of 280 square yards was divided into quarters; three of the quadrants were in full sun, the fourth was largely shaded during the morning. One of the sunny quadrants was sprayed on the underside of the leaves only, by holding the nozzle underneath the plant, pointed upward. The imperfections of such a com-

parison are obvious, but it was felt that any really important difference would be readily detected. Spraying was done in the morning with the sodium salt at 3 gm. 2,4-D/l.; 8 gallons were used—about 130 gallons per acre. Table 2 shows that the plants in partial shade and those sprayed on the underside were slightly less affected after 12 days than those sprayed on the top in full sun. Although the difference is small, it is probably significant and supports the idea that the

giving about 89 gallons per acre. The effect was much more rapid: 7 days after spraying the percentage of leaves discolored was 99 and of leaves obviously killed about 77. Later observations are discussed below.

INFLUENCE OF CARBOWAX.—The earlier experimenters in the United States used the free 2,4-D acid and employed various preparations to promote its solution in water. Of these, Carbowax 1500, a polyethylene glycol, was selected, be-

TABLE 2
INFLUENCE OF LIGHT AND SHADE
(3 gm. 2,4-D/l.)

	7 DAYS AFTER SPRAYING			12 DAYS AFTER SPRAYING		
	Sprayed on top		Sprayed on underside	Sprayed on top		Sprayed on underside
	Full sun	Shade (A.M.)		Full sun	Shade (A.M.)	
% of leaves yellowed.....	63	68	95	92	94
% of leaves killed.....	5	5	86	68	60
No. of whole plants counted..	9	7	9	8	5

killing of the leaves is at least promoted by heat effects. WEAVER and DEROSE (12) found that bean plants treated with a single drop of 2,4-D solution showed more growth inhibition in sun than in shade, which in general agrees with this conclusion. It should be noted that the figures for full sun (table 2, first and fourth columns) show very good agreement with those of table 1, which also refer to plants in full sun.

COMPARISON OF SALT AND ESTER PREPARATIONS.—An area comparable with the others and of about 150 square yards in full sun was sprayed once with a 2,4-D ester preparation (Weedone), also at a concentration of 3 gm. 2,4-D/l. Two and three-fourths gallons were used,

TABLE 3
INFLUENCE OF CARBOWAX 1500 IN SPRAY
(3 gm. 2,4-D/l. as sodium salt)

	7 DAYS AFTER SPRAYING*	
	With Carbowax (0.5%)	Without Carbowax
% of leaves yellowed..	96	65
% of leaves killed.....	18	5
No. of whole plants counted.....	11	16

* Plants in full sun.

cause preliminary experiments indicated that, although it was not needed when the sodium salt is used, it had some value as a spreader and perhaps as an adherent to the leaves. ENNIS and BOYD (2) found

that Carbowax increased the toxicity of 2,4-D on kidney beans but not on soya. Another patch of Aroma comparable with the others was therefore sprayed with 2,4-Dow (at 3 gm. 2,4-D/l.) containing 0.5% Carbowax 1500. Results

TABLE 4
APPARENT KILLING OF AROMA AFTER
1 AND 2 MONTHS

Plants of tables 1 and 2	Single spray* (%)	Two sprays, 5 days apart* (%)
<i>After 1 month:</i>		
1. Apparently killed.....	18	76
2. Doubtful but no sign of growth.....	61	16
3. Probable new growth from base.....	18	8
4. Little affected.....	3	0
<i>After 2 months:</i>		
Total % alive.....	9	2

* 2 gm. 2,4-D/l. as sodium salt.

(table 3) showed a clear increase of effectiveness, and this was borne out in later stages.

OBSERVATIONS ON APPARENT KILLING.

—Tables 4 and 5 show the state of the plants 3, 4, and 8 weeks after the treatment. The four classes mentioned in the Introduction were used. Class 2, the doubtful cases, bears too many green leaflets to be considered killed, but most of these were evidently dying as shown by the small numbers of plants apparently alive after 2 months. Table 4 shows the larger kill resulting from a second spraying with 2 gm./l. These data would be roughly comparable with the first column of table 5 except that the latter plants could not be observed after 4 weeks; between the third and fourth week some plants would pass out of the doubtful into the killed class. Taking this into account, the double spray at 2 gm./l.

appears slightly more effective than the single one at the higher concentration. As between the three lots in table 5, Weedone would appear most effective except for the condition of the new growth; it is possible that the very rapid browning of the leaves and defoliation caused by this material leads to less complete penetration to the basal growing points. On balance, the salt plus Carbowax may be considered (by a small margin) the best treatment for killing these plants.

To determine how rapidly new shoots would be expected from the base if only the tops had been killed, some intact plants, of the same type as those treated, were cut back to the base.⁴ These showed new bud development in 5–7 days and

TABLE 5
APPARENT KILLING OF AROMA AFTER
3 WEEKS AND AFTER 2 MONTHS

PLANTS OF TABLES 3 AND 4	AS SALT (2,4-Dow)*		As ESTER (WEE- DONE)* (%)
	Alone (%)	Plus Carbo- wax 0.5% (%)	
<i>After 3 weeks:</i>			
1. Apparently killed	62	87	94
2. Doubtful but no sign of growth...	30	12	2
3. Probable new growth from base	5	1†	4‡
4. Little affected...	3	0	0
<i>After 2 months:</i>			
Total % alive.....	2	1	5

* 3 gm. 2,4-D/l.

† Weak.

‡ Vigorous.

produced an average of 11.4 new branches within 2 weeks; when watered as well, the average was 18.2 branches per plant in 2 weeks. Failure to produce any new shoots in 4 weeks after treatment with

⁴ Observations kindly made by Dr. KEVORKIAN.

2,4-D is therefore very strong evidence of death.

Trials on a larger scale, using 50 gallons at a time, have been carried out with the same preparations on plants of about the same size and also some which had not been cut back and were 10-12 feet in height. The results were very much as above. After 2 months a few plants in the large-scale experiments showed sprouting from the roots, not at the base but at some distance from the treated parts; in

dersides, although stomata are limited to the underside, agree with this finding. Nevertheless, it was thought worth while to investigate the stomatal opening of *Aroma* for the following practical reason: the stomata may not provide the only path, but they must certainly provide the most rapid path of entry. If, therefore, the plants are sprayed during stomatal closure and subsequently exposed to heavy rain, much of the spray may be lost before penetration. Spraying at the

TABLE 6
VARIATION OF STOMATAL OPENING IN
AROMA WITH TIME OF DAY

TIME	PERCENTAGE OF STOMATA				TOTAL FRAC- TIONAL OPENING
	Closed	Nearly closed	Well open	Fully open	
7:00 A.M.*	20	10	20	50	0.66
9:30.....	0	15	14	71	.84
1:15 P.M....	0	0	11	89	.97
2:45.....	50	20	10	20	.31
3:45.....	61	28	11	0	.14
4:00.....	38	42	16	4	.24
6:15†.....	23	31	38	8	0.41

* Twenty minutes after sunrise; clouds.

† Sunset.

other words, the material was not well transported along the extended and woody horizontal roots. This type of new growth has not yet been observed on the smaller plots described above.

OBSERVATIONS ON STOMATAL OPENING.—It was shown in table 2 that plants sprayed on the underside of the leaves showed no increased susceptibility. Since the proportion of stomata on the undersides and upper sides of *Aroma* leaves is about 5:1, this suggests that the stomata do not play a major part in penetration of the spray. The negative results of WEAVER and DEROSE (12), who similarly found no difference in the growth inhibition (not killing) of nasturtium when sprayed on the upper sides and un-

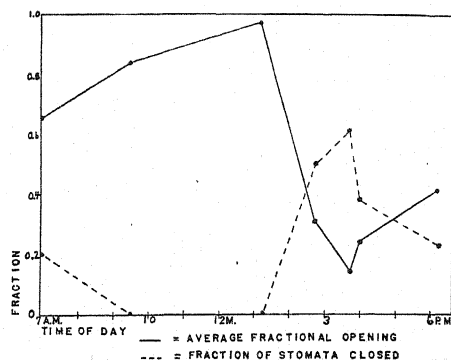


FIG. 1.—Stomatal opening of *Aroma* in Atkins Garden on clear days. Solid line indicates "average fractional opening" (see text); dotted line gives fraction of stomata which were fully closed. March, 1947.

time of maximal stomatal opening would therefore be the safest procedure.

The condition of the stomata was examined by the peel method (5), using plants growing in the open in full sun. The leaflets were cut in half, and with fine tweezers fragments of the upper and lower epidermis were quickly stripped off and dropped into absolute alcohol. This procedure was used by LOFTFIELD (6), in his extensive field studies on this subject, with good results. The peels were examined later under the microscope and assigned to arbitrary classes as to extent of opening. Observations were made on three successive days, but with the exception of the 7:00 A.M. reading, the sky was clear throughout and the weather dry.

The results (table 6, fig. 1) show that the stomata open rapidly after sunrise and close sharply in the afternoon, to open again, at least partially, in the evening. The fractional opening given in the last column of table 6 is an approximate figure obtained by arbitrarily assigning the numerical values of 0, 0.2, 0.7, and 1.0, respectively, to the stomata in the four classes given. Such behavior is similar to that recorded for other plants in dry weather—*Verbena* and *Fouquieria* (5), alfalfa (6), and orange (8)—although almost every possible pattern of opening and closing has been recorded. From the

hillside pasture. Three gallons were used on about 3000 square feet which was, of course, by no means completely covered with Guao; this amount sufficed to wet all the leaves.

Table 7 compares the two treatments. The classification used differs from that for Aroma, but, since these plants are more compact and have fewer leaves, it was easier to obtain an idea of the extent of damage than with Aroma. If we take the first two classes together, it is evident that the free acid (although its concentration was 20% lower) is at least twice as effective as the salt on these plants.

TABLE 7
EFFECT (PERCENTAGE OF PLANTS) OF SINGLE APPLICATION OF 2,4-D AS SALT,
FREE ACID, OR ESTER ON GUAO

	3 WEEKS		5 WEEKS		8 WEEKS		ESTER		
	Salt	Acid	Salt	Acid	Salt	Acid	1 week	3 weeks	6 weeks
1. Apparently killed.	2	22	15	51	22	49	42	83	75
2. Seriously injured or dying. . . .	15	22	16	23	24	18	47	3	2
3. Somewhat damaged.	36	26	36	21	30	18	11	10	13
4. Apparently intact.	47	30	33	5	24	15	2	3	10

viewpoint of spraying for penetration, the results indicate that early morning (after dew has dried) would be optimal.

GUAO

The problem with Guao is somewhat different. The leaves of this plant are glabrous and shiny, indicating that penetration would be difficult. Further, they hang almost in a vertical plane, so that ordinary sprays run off quickly without time to dry on. To overcome the penetration difficulty, a spray was made up using the free 2,4-D acid with 0.5% Carbowax; the maximum that could be put into solution in this way was 2.3 gm./l. This was compared with the sodium salt at 2.8 gm./l. (i.e., 2,4-Dow 4 gm./l.) in two comparable plots, in full sun on a stony

Since it is inconvenient to make up solutions of the free acid in high concentration, other preparations were used. It was found that Weedone (ester form), because of its oily nature, adhered to the leaves, and, instead of running off, hung in a sticky drop at the tip of the leaf. Table 7 shows that, as with Aroma, its effect is much more rapid than that of the salt or acid, the percentage of plants apparently killed being greater in 1 week than with the other treatments in 3 weeks. The later observations also show much greater toxicity of Weedone. After 6-8 weeks, both above-ground parts and roots were soft and apparently rotting in the plants of class 1, so that these can be considered as killed, in spite of the fact that all these plants were propagated

from stumps of several years' growth and had heavy and woody stems.

The apparent slight improvement of the plants at the 8-week count is probably due to sampling error. Not less than 250 plants were counted for each determination, but the assignment to the four classes is obviously open to some variation.

In the absence of a more extensive investigation it is clear that this plant can be at least partially eradicated by the ester type of spray.

Summary

1. The herbicidal effectiveness of 2,4-dichlorophenoxyacetic acid (2,4-D) has been tested on Aroma marabu (*Dichrostachys nutans*) and Guao (*Comocladia dentata*), two perennial and highly resistant woody weeds of Cuba.

2. As judged by criteria based on rapid defoliation and on ultimate apparent killing, a single spraying with 2,4-D in the sodium salt or ester form, at a con-

centration of 3 gm./l., or two sprayings 5 days apart at 2 gm./l., were almost completely herbicidal on Aroma.

3. Little, if any, regrowth of basal buds took place within 2 months of spraying, although controls which were cut back regenerated rapidly. There was some evidence of new growth from horizontal roots, however.

4. Addition of 0.5% Carbowax definitely increased the toxicity of aqueous 2,4-D sprays.

5. With Guao, ordinary sprays were of only moderate effectiveness, but Weedone gave at least a 75% kill within 6 weeks.

6. Some of the difficulties involved in assessing toxicity on woody plants are discussed.

7. The influence of stomatal opening and other conditions was briefly investigated.

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SEEDLING ANATOMY OF BRACHYPODIUM DISTACHYUM

NAOMI MULLENDORE

Introduction

The anatomy of the grass seedling has been studied for more than two centuries, but there still remain some questions concerning the vascular pattern of the first few internodes and its relationship to the developing leaves and stem. In some grasses the shortness of the internodes, which results in the internodal pattern being influenced by that of the nodes, and the great number of vascular bundles have increased the difficulty of understanding the situation. In *Brachypodium distachyum* (L.) Beauv. all the internodes, even the first one, are long, the diameter of the stem is small, and the vascular bundles are few. These characteristics are advantageous for study in that a simpler vascular pattern results. This paper is an attempt to add to the clarification of some of the problems of anatomy of the grass seedling.

Material and methods

Seeds of *B. distachyum* were obtained in 1943 from Dr. PAUL WEATHERWAX of Indiana University. Dr. WEATHERWAX had received seeds some years earlier from La Mortola, a botanical garden in Ventimiglia, Italy, where the plant grows wild as a weed. He planted them in the greenhouse at Indiana University, and plants have been grown there since that time.

The grains were sterilized in Semesan dust, soaked in boiled distilled water a day, and then allowed to germinate. Some were planted vertically in pots; other were arranged in parallel rows in sterilized Petri dishes on moistened filter paper and allowed to germinate in a vertical position in diffused light. To secure

various stages in germination, seedlings were removed from time to time and killed and preserved in FAA.

The preserved seedlings were infiltrated, using *n*-butyl alcohol, and imbedded in a rubber-paraffin-beeswax mixture. Transverse and longitudinal sections of various stages of development were stained in a modified triple or quadruple stain.

Observations

The embryo consists of a thick, somewhat flattened, cotyledon, a plumule covered by the coleoptile, an epiblast, and the hypocotyl and radicle which are covered by a sheath, the coleorhiza (figs. 1, 2). There is no vascular tissue in the epiblast or in the coleorhiza. These structures are apparently flaplike extensions of the cotyledon, forming a sheath around the axis and extending downward to cover the radicle (figs. 2, 3). In *Avena* the coleorhiza and the epiblast are considered to be outgrowths from the cotyledon or axis or both and of little morphological importance (1, 6). *Brachypodium* is apparently similar. In development its primary root pushes through the coleorhiza which is left as a lobed collar at its base (fig. 3).

The coleoptile arises at some distance above the cotyledon but on the same side of the axis. The first true leaf arises above it on the opposite side. A bud usually occurs in the axil of the coleoptile (fig. 4). AVERY (2, 3) has reported that a bud is almost universally present in the axil of the coleoptile of oats but is rarely present in that of maize. In *Brachypodium* several adventitious roots arise above the divergence of the coleoptile.

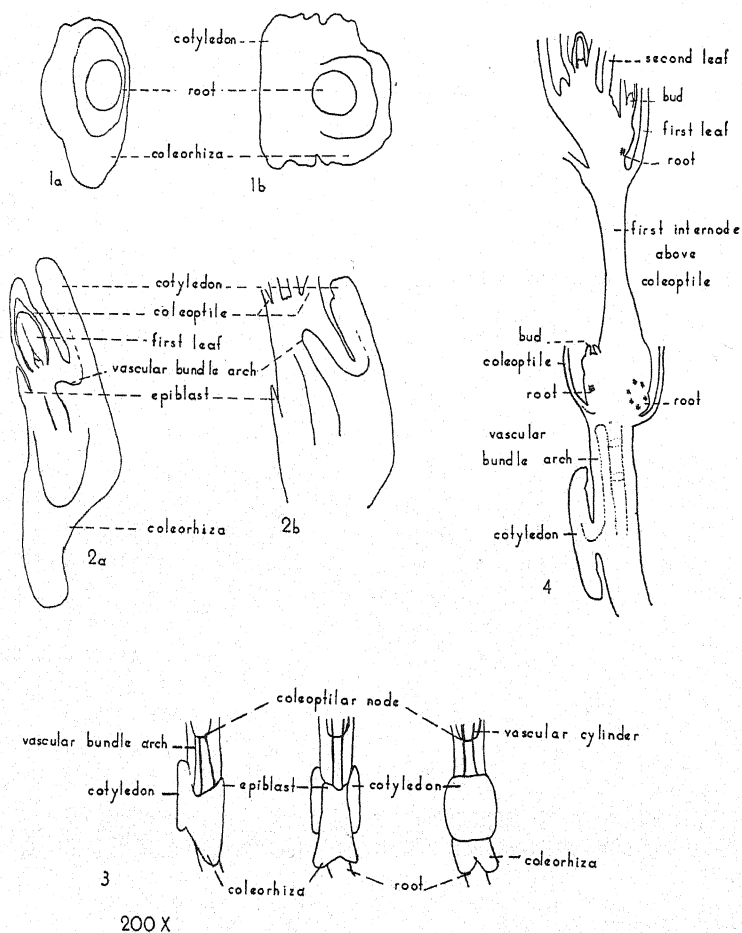
The vascular tissue to the cotyledon

runs upward in the stele of the first internode to the divergence of the coleoptile at the first node above the cotyledon, thence downward as a vascular bundle in the cortical tissue parallel to the stele, and then into the cotyledon and upward again (figs. 2, 3, 4, vascular bundle arch). Similar development has been reported for *Avena* (3, 4, 6).

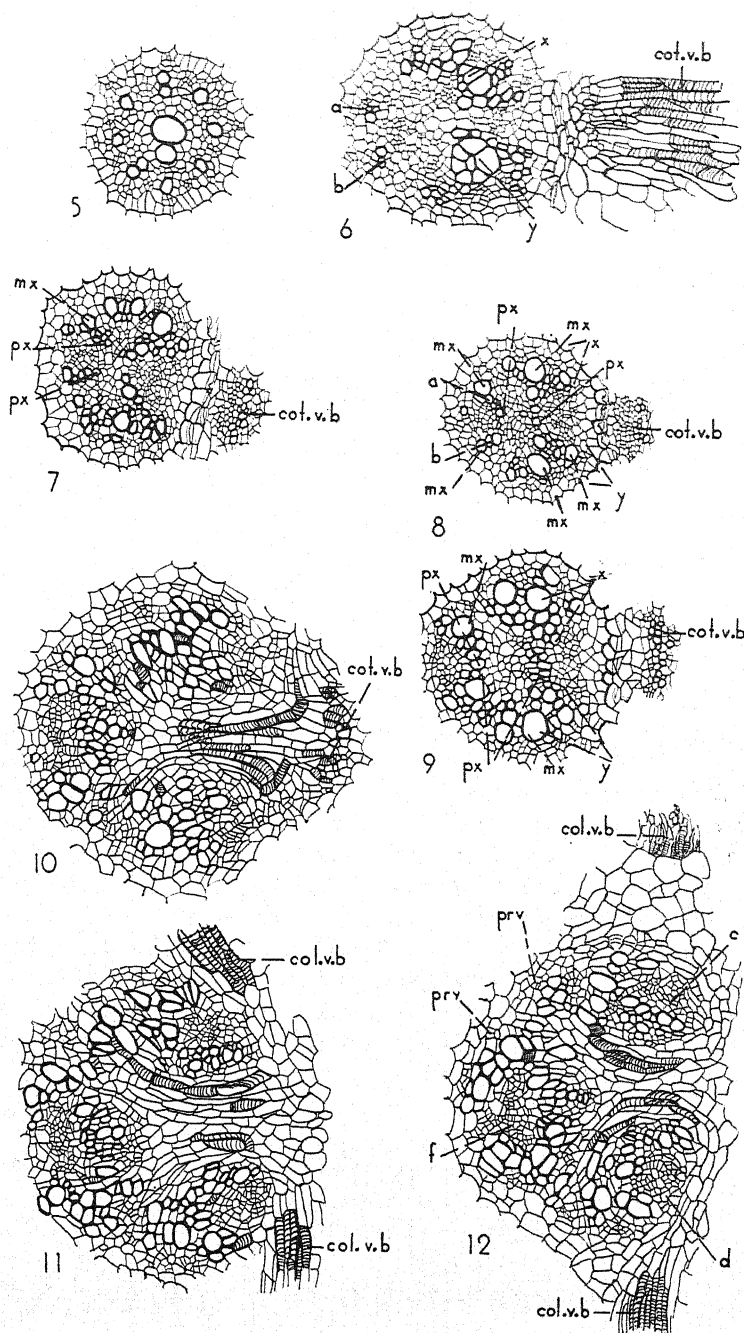
The primary root has a radial stele, usually with seven or eight protoxylem points (fig. 5). Transition occurs largely in the first internode, the so-called meso-

cotyl—the internode above the attachment of the cotyledon—and is somewhat difficult to follow in the region near the divergence of the coleoptile because of adventitious roots and the bud in the axil of the coleoptile. All drawings of transverse sections have the same scale of magnification.

The vascular tissue in the lower part of the transition region is largely in two groups, but one is larger (fig. 9). At the lower level two xylem strands, which in the root were opposite the divergence of



FIGS. 1-4.—Fig. 1, transverse sections of embryo; (a) below attachment of cotyledon; (b) through cotyledon. Fig. 2, reconstructions of longitudinal sections of embryos. Fig. 3, different views of middle portion of young seedling. Fig. 4, idealized reconstruction of longitudinal section of seedling.



FIGS. 5-12.—Series of transverse sections of stele of seedling. Fig. 5, primary root. Figs. 6-9, stages in transition of first internode. Fig. 10, at level where cortical cotyledonary bundle diverges from vascular tissue of axis. Fig. 11, at divergence of coleoptilar bundles from axis. Fig. 12, above level where coleoptilar bundles have diverged, axis has three main vascular bundles plus small provascular strands external to these (*px*, protoxylem; *mx*, metaxylem; *cot. v. b*, cotyledonary vascular bundle; *col. v. b*, coleoptilar vascular bundle; *a, b, x, y*, xylem strands; *c, d*, vascular bundles).

the cotyledon, show a rotation of approximately 90° (fig. 6, *a, b*). At a higher level further rotation results in a union of these two protoxylem points internally and in an extension of the two metaxylem arms at right angles from this union, the U-shaped xylem so formed embracing the phloem (figs. 7, 8, *px, mx*). This vascular group becomes one of the three main vascular bundles of the upper part of this internode (figs. 12, 13, *f*).

At the lower level of the first internode the other five, or six, protoxylem strands from the root also are rotated about 90° and, with the metaxylem, are united into two groups (fig. 6, *x, y*) extending at right angles to the cortical cotyledonary bundle, which lies in the cortex opposite the middle of the cotyledon (figs. 7, 8, *cot. v.b*).

At a higher level, the protoxylem now further rotated, these two groups apparently fuse internally (figs. 8, 9, *x, y*). This forms the second, larger vascular group of this internode. The arrangement is complicated by the extra protoxylem and metaxylem which lie on each side of this group, extending laterally toward the other group.

At the upper end of the internode the vascular group on the cotyledonary side of the axis is divided, the middle portion turning downward and becoming the cortical cotyledonary bundle (fig. 10, *cot. v.b*); the parts on each side immediately adjacent to this middle part diverge laterally to become the coleoptilar bundles. These turn upward, on opposite sides of the axis, into the coleoptile (fig. 11, *col. v.b*). These bundles may be seen in older seedlings made somewhat transparent by FAA (fig. 3). The coleoptile has only these two vascular bundles, which lie opposite each other, in lateral positions. The lateral portions of the main bundle remain as two vascular

bundles of the axis (fig. 12, *c, d*) and continue into the next internode.

At the divergence of the coleoptile these two bundles of the axis, plus the one bundle opposite the middle of the coleoptile, form the three bundles of the lower part of the next internode of the axis. The extension of the metaxylem in this region forms what are practically amphivasal bundles. In addition, there are small external provascular strands, probably the downward extensions from leaves above (figs. 12, 13, *c, d, f, prv*).

At a higher level the vascular strand which was opposite the middle of the cotyledon (fig. 13, *f*) forms one large bundle which will diverge as the midrib of the next leaf above—the first true leaf—and a small bundle on each side of it (fig. 14, *g, h*). The other two bundles (fig. 13, *c, d*) form three large bundles and two small bundles (fig. 14, *c, d, e, i, j*).

Two adventitious roots are shown (fig. 14, *k, l*) beginning to develop from the axis opposite each other and at right angles to the bundles which will be the midribs of the first and second leaves above the coleoptile. Just above these a bud develops in the axil of the coleoptile (fig. 15, *bud*).

Examinations of a number of seedlings were made, and all were found to have a similar development. Further development of the upper part of the axis will be illustrated with an older seedling having six leaves.

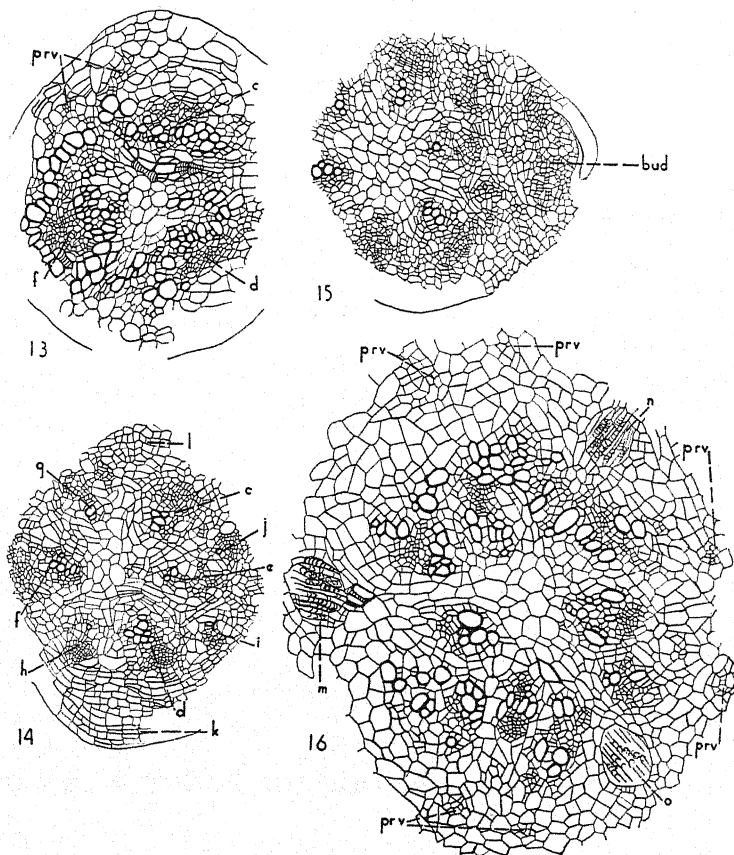
In such an older seedling, in the second internode, additional vascular bundles are found, lying slightly external in the stele. Apparently these represent downward extensions from the leaf above, which end blindly in the cortex or join up externally with the outer part of the vascular ring.

In the upper part of the internode, just below the divergence of the first true

leaf, three large bundles, at approximately one-third of the circumference apart, elongate radially and diverge out of the circle (fig. 16, *m, n, o*). At the divergence of the leaf the radially elongated bundle forming the midrib divides, the smaller inner portion remaining as one of the bundles of the central cylinder. At a higher level this branches to form the midrib of the second leaf above, i.e., the third true leaf. The other two bundles which elongate radially and diverge from the central cylinder also leave small strands in the central cylinder. In the

cortical region, in addition to these three main bundles of the leaf, there are two small provascular strands in each of the spaces between them (fig. 16, *prv*). These small bundles are the lower extensions of veins from the leaf and are the bundles which unite externally with the central cylinder at a lower level.

The leaf has nine vascular bundles at its divergence from the axis, but at this level very little xylem is differentiated in the six smaller ones. The leaf bundles have sclerenchymatous caps in addition to the bundle sheaths.



FIGS. 13-16.—Transverse sections of stele in second internode of seedlings. Fig. 13, just above divergence of coleoptile. Fig. 14, at level of origin of two adventitious roots. Fig. 15, axis and bud in axil of coleoptile. Fig. 16, just below divergence of first true leaf (*prv*, provascular strand; *c, d, e, f, g, h, i, j*, vascular bundles of axis; *k, l*, adventitious roots; *m, n, o*, vascular bundles of axis which continue as main vascular bundles of first true leaf).

Just above the divergence of the first true leaf, two adventitious roots arise from the axis. The axillary bud arises above these roots (fig. 17, bud).

The vascular bundles to the bud come largely from two smaller bundles which lie on each side of the bundle forming the midrib of the leaf. These bundles branch, and divergences from them enter the bud. Additional supply comes from lateral strands of the main bundle lying between these smaller ones, the bundle whose branch formed the midrib of the leaf. There is considerable anastomosis at the node, as evident from horizontal extensions of vascular tissue in this region where adventitious roots and the axillary bud are developed.

In the region where the bud in the axil of the first true leaf arises, the bundles of the axis show a greater development of the bundle sheath and are set apart more clearly, having the appearance of true stem bundles (fig. 18).

In this internode just above the divergence of the bud there are twelve main axial bundles, six larger alternating with six smaller, and still smaller bundles lying somewhat externally to these main bundles. These smallest bundles are downward extensions from leaves above.

Below the divergence of the second true leaf, five of the main bundles elongate radially and diverge outward to form the five bundles with lignified xylem found in that leaf at its divergence. These five bundles are the midrib, two large laterals, which occupied areas about one-third of the circumference of the vascular ring, and two new bundles located near the margin of the leaf. The latter bundles are continuous with small bundles which remain on each side of the bundle which is to be the midrib of the fourth leaf. In addition to the five larger bundles with lignified xylem, there are six less differ-

entiated ones, making a total of eleven in the second leaf at its divergence. All these bundles have sclerenchymatous caps.

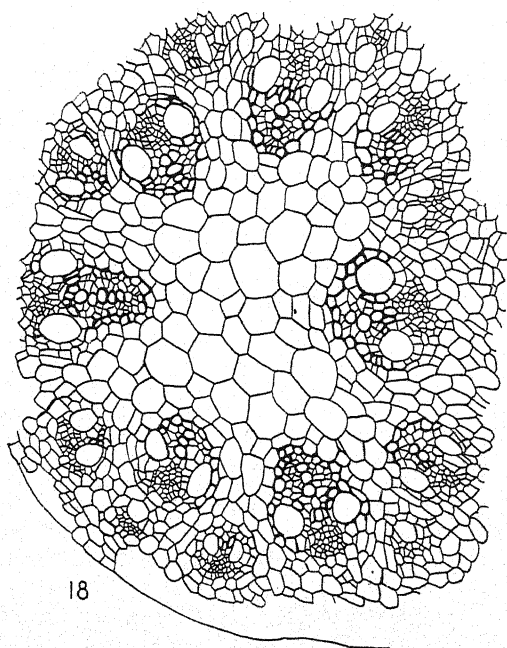
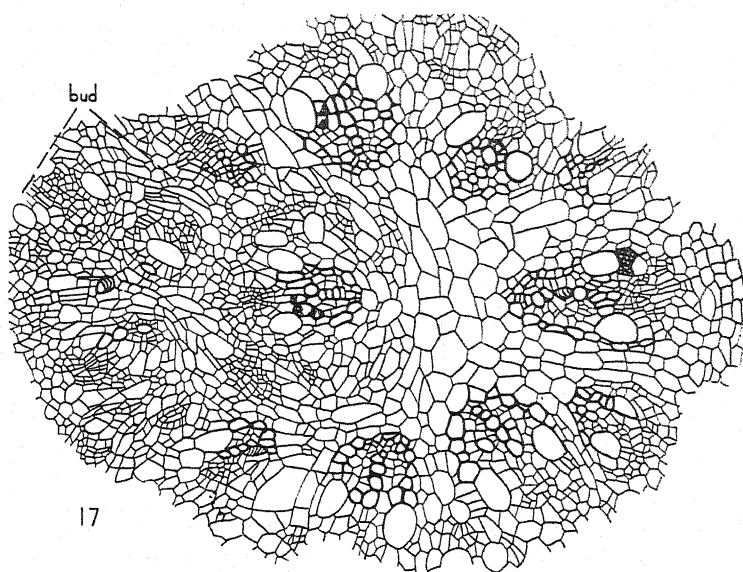
At a higher level in the leaf the number of vascular bundles is increased to thirteen by the appearance of two small ones.

Above the divergence of the second true leaf eight main vascular bundles, four larger, are present in the central cylinder. In addition, there are horizontal extensions of provascular strands. These apparently are partly related to the axillary bud.

The vascular bundles in the axillary bud are very immature and enter the central stele by horizontal branches, uniting eventually into two horizontal strands, one on each side of the vascular strand which is to diverge as the midrib of the fourth leaf.

The origin of the third leaf is similar to that of the second, but in this younger leaf there seems to be no division of the radially elongated bundles to leave vascular strands in the axis, which means that the bundles from the leaf above have not differentiated this far down. The new bundle for the midrib of the fifth leaf and the vascular tissue for the axillary bud unite with the provascular strands lying on each side of, and inward from, the bundle which forms the midrib of the third leaf.

At its divergence the third leaf has five vascular bundles with lignified xylem and nine provascular strands. There is a very young bud in the axil of the leaf. The fourth leaf is embryonic, but eleven provascular strands can be seen at its divergence. The number of bundles in this leaf is increased at a higher level to nineteen. In all except five of these, however, the conducting tissue is provascular. The additional bundles have developed in the



FIGS. 17-18.—Transverse sections of stele where axillary bud of first true leaf arises and just above level where leaf has diverged from axis.

leaf and have not joined with those of the axis or with other veins of the leaf.

In the seedling described six true leaves have diverged from the axis. The fifth leaf has five provascular strands where it diverges from the axis; at a higher level it has seven provascular strands, so apparently they differentiate first in the leaf. Above the fifth leaf there are only three provascular bundles in the axis. These diverge into the sixth leaf, and the stem tip is meristematic without any vascular tissue.

In the immature region near the stem tip the vascular bundle development varies somewhat; this may be explained by the embryonic condition of the axis and the failure of complete extension of vascular tissue. In the embryonic region the three main leaf bundles diverge without leaving traces which remain in the central axis, as is true in the lower older leaves and axis. This would seem to indicate that the vascular bundles arise in the leaves and then extend into the axis. This evidence supports the explanation by GRÉGOIRE (5) that the procambial strand of a leaf trace first appears in the base of the leaf primordium and then de-

velops upward into the leaf and downward into the stem, where it joins the system of bundles coming down from other leaves.

Conclusion

Evidence from a study of the seedlings of *Brachypodium distachyum* indicates that the vascular tissue of the stem is developed as a downward extension of vascular bundles from the leaves. As more leaves are developed, the vascular bundles of the stem increase in number, since these leaf traces may extend through several internodes and since the succeeding leaves are larger and have more vascular bundles.

A vascular trace to a leaf may be related to vascular tissue above by a convergence of an upper vascular strand with the leaf trace to form an axial vascular bundle at a lower level.

The author is grateful to Dr. PAUL WEATHERWAX for helpful suggestions and criticisms concerning the writing of this paper.

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PROTHALLI OF CERATOPTERIS THALICTROIDES

T. S. MAHABALÉ

Introduction

Ceratopteris is a monotypic genus of the family Parkeriaceae, widely distributed in the tropics of the Old as well as the New World. It is generally believed to consist of a single species, *Ceratopteris thalictroides* Brongn. (10), but this view is not shared by all authors. BENEDICT (3), for example, has split the genus into four species, *C. thalictroides*, *C. lockharti*, *C. pteroides*, and *C. deltoides*, basing the distinction on the form of the annulus, the presence or absence of the lip, and the number of the spores per sporangium. *C. thalictroides* occurs as an annual or perennial fern in India and Ceylon in an amphibious state on the margins of pools and river banks and is also seen floating in some deep-water reservoirs in Bengal, Assam, and other parts of India.

The prothalli of this fern were studied by KNY (18) and by LEITGEB (20) from material obtained by germinating the spores under laboratory conditions. In October, 1938, the writer found some prothalli of this plant near a thicket of sporophytes on the banks of the Sabarmati River at Ahmedabad, Western India. On examination these were found to be somewhat different from those described by previous authors, and hence it was thought worth while to describe them from their natural habitat.

Observations

GENERAL STRUCTURE.—The adult prothallus measures 5×4.2 mm. and consists of two subequal or sometimes unequal lobes with the meristem lying in the notch (figs. 1, 2). The formation of such unequal lobes is also known in *Pteris longifolia*, *P. biaurita* (22, 23, 25),

Acrostichum aureum, *Ceropteris* (*Pityrogramma*) *calomelanos* (13), *Cibotium schiedei*, and *Asplenium caudatum* (18).

In a typical adult prothallus, however, the two lobes are more or less equal (fig. 1). The connection of the primary filament could be noticed in some prothalli up to an unusually late stage, when they had already formed the reproductive organs. The dorsal surface of the prothallus is green, and on its ventral surface there is a short cushion, about five cells thick, which bears rhizoids at its posterior end and the archegonia at the anterior (figs. 2b, 3). The wings are single-layered and bear a large number of antheridia all along the margin, including the portions near the notch (figs. 1-3). All the prothalli were bisexual. In spite of a careful search no one-lobed prothalli bearing only antheridia, like those described by KNY (18), were found. There was a marked tendency toward dioecism in the material studied by KNY which might have resulted from the fact that his material was obtained from culture; or it may be that the male prothalli are very short-lived in their natural habitat.

REPRODUCTIVE ORGANS.—The form and development of the reproductive organs (figs. 4-13) agree with KNY's description (18). The development of the antheridium is of the *Aneimia* type (figs. 4-9). Unlike the antheridia of most polypodiaceous ferns, those of *Ceratopteris* lie imbedded in the marginal tissues of the prothallus (figs. 1, 3, 9). A vertical section shows seven to eight spermatocytes (figs. 7, 8). The antheridium opens by the partial disintegration of a cover cell, as in *Aneimia* and some polypodiaceous ferns (fig. 9). The archegonium is of the usual

leptosporangiate type (figs. 10-13). The axial row consists of a three-nucleate neck canal cell, one ventral canal cell, and the egg. The neck cells are in four tiers of about five cells each (fig. 13).

EMBRYO.—The earlier stages in embryogeny agree with those in the Polypodiaceae (figs. 14-16). KNY (18) thought that both the anterior quadrants participate in the formation of the leaf from

did not give any definite opinion. Recently HOWE (17) studied this point in young plants raised from the leaf buds and found that the first adventitious root at the first node develops from a hypodermal cell derived from the cell immediately below the leaf initial. Later roots on a young plant may develop from the cells derived from the leaf initial itself and are also of hypodermal origin. The secondary roots arise from certain enlarged cells of the inner cortical layer of the primary root situated near its apical cell. Accord-

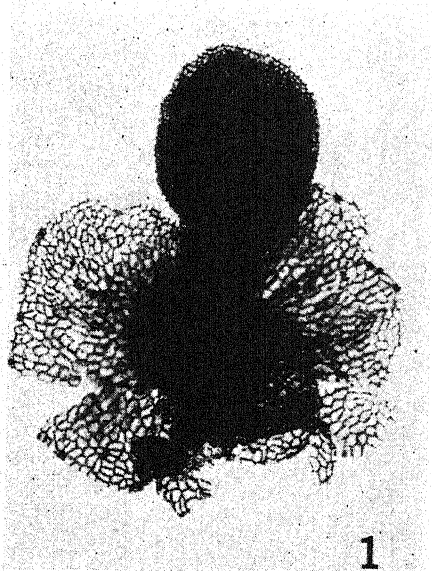


FIG. 1.—Prothallus with young sporophyte. $\times 25$. Note antheridia at margin.

which the stem arises afterward as a lateral bud. He compared this condition with that in the monocotyledons, in which also a single cotyledon is formed by the anterior quadrants. From this he further suggested a polyphyletic origin of the angiosperms through other vascular cryptogams such as *Isoetes*. This view, however, is no longer tenable.

KNY (18) also thought that the secondary roots arising from the nodes at the bases of petioles in the first-formed leaves are exogenous in origin. FORD (12), who studied the anatomy of the plant,

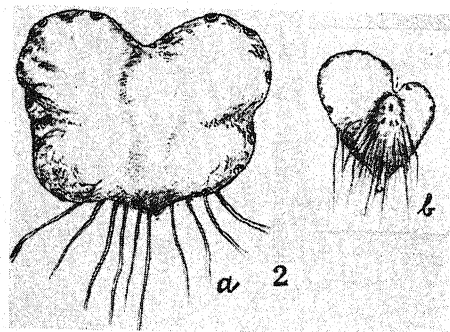


FIG. 2.—Two adult prothalli. $\times 22$. a, dorsal surface of prothallus. b, ventral surface, showing rhizoids and small cushion. Note unequal lobes.

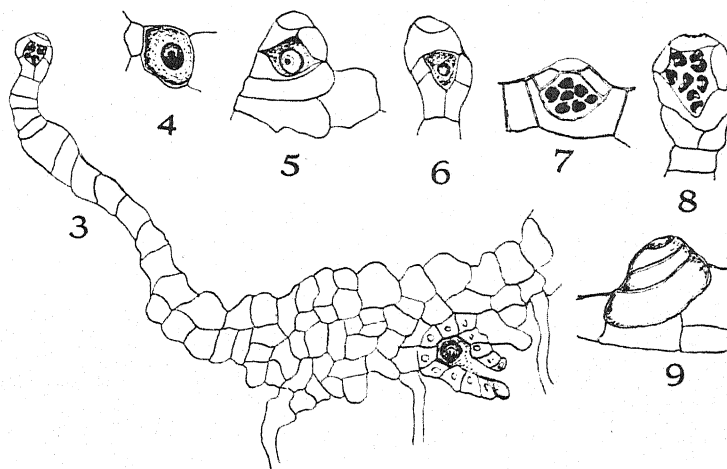
ing to HOWE, this layer finally occupies the position usually taken by the endodermis. Subsequent behavior of the secondary root initial is the same as that of the primary root initial. In the embryo shown in figure 17, a secondary root (*Sr*) is arising from the primary root (*R*). The leaf at the base of which it appears to arise is quite advanced, but, since the stem region is not yet well differentiated, the endodermis is not well defined. It is from such cells, however, which later form the endodermis, that the secondary root originates; and, therefore, it should be considered to be endogenous and not exogenous. This was also the view of VAN TIEGHEM, DOULIOT, and POIRAULT (as cited by FORD [12]).

MYCORRHIZA.—The prothallus occasionally harbors some fungal filaments in the cells of the cushion on the lower side. A similar fungus was noticed in the prothalli of this plant by DE BARY (9), who considered it to be a species of *Pythium*, and also by LEITGEB (21), who believed it to be a species of *Completozia*. In the present study, to judge from the appearance of the fungus in the host cells, it seems to be the latter. A similar

those of the prothallus and sporophyte and occur in other ferns also. The exact origin or significance of such "*Mittelbildungen*" are still unknown in most cases (McVEIGH [24]).

Discussion

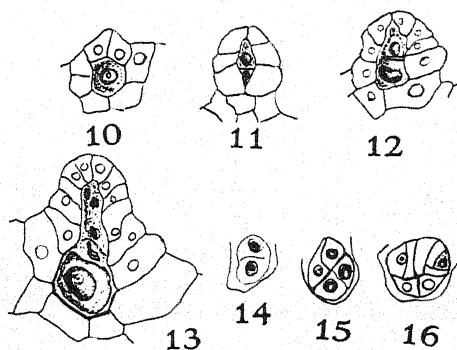
The systematic position of the family Parkeriaceae to which *Ceratopteris* belongs is not yet clear. DIELS (10) placed it immediately after the Polypodiaceae,



FIGS. 3-9.—Antheridia. Fig. 3, vertical section of prothallus showing archegonium on ventral surface and antheridium at margin. $\times 65$ (approx.). Figs. 4-6, development of antheridium. $\times 100$. Fig. 4, antheridium initial. Figs. 5-6, young antheridia. Fig. 7, antheridium with eight spermatocytes. Fig. 8, the same, a more advanced stage. Fig. 9, entire antheridium which has discharged its contents.

fungus was noticed in the prothalli of *Botrychium virginianum* by CAMPBELL (7) and in the prothalli of *Ophioglossum fibrosum* by the author.

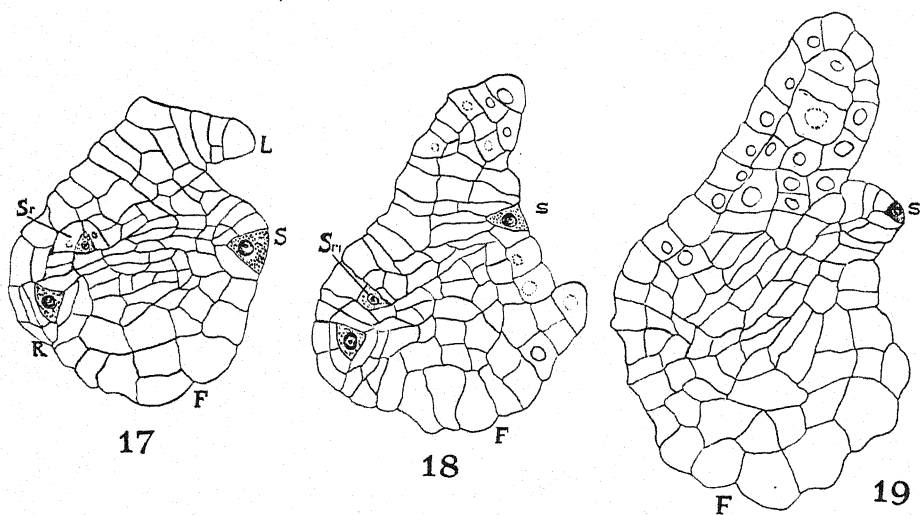
ABNORMALITIES.—In one case stomata were seen on the wings of the prothallus similar to those described by GOEBEL (14) for the prothalli of this species. GOEBEL obtained the prothalli by cultivating the leaves of juvenile sporophytes in moist conditions after decapitating the apex of the shoot. Similar examples of prothallial regeneration in this species have been reported by BALLY (1), KOEHLER (19), and BEYERLE (4). They show characters intermediate between



FIGS. 10-16.—Figs. 10-12, development of archegonium. $\times 100$. Fig. 13, mature archegonium. $\times 100$. Figs. 14-16, successive stages in early embryogeny. $\times 62$.

whereas CAMPBELL (7) put it after the Cyatheaceae. BOWER (6) considered that the genus *Ceratopteris* is more closely related to the relatively primitive Gymnogrammoid ferns, such as *Cryptogramme* and *Onychium*, rather than to the advanced types like *Adiantum* and *Cheilanthes*. CHRISTENSEN (8) supported this view and placed it under a separate group of Gymnogrammoidae, the Ceratopteridae. In view of the lack of any

the prothallus is often suppressed. The prothalli of the more advanced Gymnogrammoid ferns, like *Adiantum* and *Cheilanthes*, are also not comparable with those of *Ceratopteris* on account of some aberrant features noticeable in them (15, 16), such as collenchymatous thickenings in the region of the cushion—as those in *Aneimia* and *Lygodium* (2, 15, 27)—or the tracheids sometimes found in the prothalli of some species of *Adiantum* (22).



FIGS. 17-19.—Three advanced embryos. F, foot; L, leaf; R, root; S, stem; Sr, secondary root; Sri, secondary root initial. $\times 125$.

general agreement on the question, it seems worth while to see what light a comparison of the prothalli of *Ceratopteris* with those of allied genera can throw on the subject.

According to BOWER (6), the Gymnogrammoid ferns are related to the Osmundaceae through the Plagiogyriaceae. The prothalli of some primitive Gymnogrammoid ferns, such as *Gymnogramme*, *Anogramme*, *Hemionitis*, and *Ceropteris*, are known. These are essentially of the leptosporangiate type. In them the archegonia are borne on certain tuberous outgrowths, and one of the two lobes of

The prothalli of the Osmundaceae resemble those of *Ceratopteris* in having marginally or terminally distributed antheridia. The prothalli of these forms, however, differ from one another in many other respects, such as the plane of the first wall in the antheridial cell, which is flat in *Ceratopteris* but curved in *Osmunda*; the mode of dehiscence of the antheridium, which takes place by the overthrow of a special opercular cell in *Osmunda* but not so in *Ceratopteris*; the position of antheridia, imbedded in *Ceratopteris* but not so in *Osmunda*; etc. The sunken condition of the antheridia in

Ceratopteris is comparable with that in the Marattiaceae, Ophioglossaceae, Lycopodiaceae, and Equisetaceae; but it should be remembered that it is also known to occur in some Polypodiaceae, such as *Dryopteris stipularis* (5) and *Nephrodium molle* (11) and in a Cyatheaceous fern, *Lomaria quadripinnata* (26). As a matter of fact, in their mode of dehiscence the antheridia of *Ceratopteris* resemble those of *Plagiogyria*, *Aneimia*, and *Mohria*, but the prothalli of these genera show considerable difference in their shape—being lopsided in *Aneimia* and *Mohria*, on account of the lateral meristem, and cordate in *Ceratopteris* and *Plagiogyria* (15), on account of the meristem lying in the notch.

It thus appears that the prothalli of *Ceratopteris* are not closely comparable with those of any allied group, and, therefore, no definite conclusions can be arrived at from this source. For the present it seems best to adopt BOWER's (6) view that the genus *Ceratopteris* is a relative of the Cryptogrammeae adapted to aquatic life.

Summary

1. The prothalli of the amphibious fern *Ceratopteris thalictroides* Brongn. are hermaphroditic and cordate. The antheridia, of the *Aneimia* type, are imbedded in the tissues of the prothallus and are marginally or terminally distributed.

The number of spermatocytes in an antheridium is small, about eight occurring in a vertical section. The archegonia are of the Polypodiaceous type and the early embryogeny is normal.

2. The leaf shows a precocious development, and the stem quadrant is rather slow in differentiation, probably owing to the aquatic habit of the plant. The origin of the primary root is normal. The secondary roots arise from the endodermal cells of the primary root and are therefore endogenous. No hairs of any kind are found on the prothallus.

3. One specimen showed stomata similar to those observed by GOEBEL (14). A facultative fungus, presumably a species of *Completozia*, was noticed in some prothalli.

4. A comparison is made of the prothalli of *Ceratopteris* with those of the primitive Gymnogrammoid ferns, but no final conclusions on their affinities can be drawn for want of adequate data.

The writer wishes to thank Professor P. MAHESHWARI for his kindness in going through the manuscript and giving helpful suggestions; Professors J. J. ASANA and R. N. SUTARIA for the use of facilities with which to work; and Dr. S. P. AGHARKAR for providing some literature.

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A COMPARATIVE STUDY OF THE NUMBER AND LENGTH OF ROOTS PRODUCED IN NINETEEN ANGIOSPERM SPECIES

HOWARD J. DITTMER

Introduction

Quantitative studies of the total root development of a few plants have been reported by PAVLYCHENKO (6), DITTMER (4), and WEAVER and ZINK (8, 9). In these studies definite counts were made of the roots arising from the base of the plants, and in most cases a record was kept of all the roots in the different subdivisions as main, secondary, tertiary, etc.

Investigations of this nature give an excellent indication of a plant's soil-binding efficiency (3) and may also be used, along with length and diameter studies of the root systems, to determine the absorptive and conductive capacity of plants. KRAMER (5) has pointed out the importance of root extension and rate of growth in making sufficient water available for the plant's needs without depending upon capillary movement.

Material and methods

The plants used in this study were collected in New Mexico while growing naturally on the edges of gardens and lawns. Collections were made in July and August, when the plants were mature and in flower with the exception of *Ulmus pumila*, for which the specimens were 4-6 years old. The reaction of the caliche soils in which they were growing varied from pH 8.0 to 8.4. Entire root systems were collected in so far as possible by digging around the plants with a long-bladed spade and then carefully removing a large cylinder of soil containing the roots. With a few of the larger species, as *U. pumila*, the entire root systems were not obtained, but a sufficient number of plants was taken to insure accurate counts and measurements of all parts.

After a plant was collected, its root system was carefully washed to remove

soil clinging to the various parts and also to free one plant from another. Whenever possible, counts and measurements were made immediately on the root systems. When this was not possible, the material was preserved in a weak solution of formalin for later study. The method used in making the counts and measurements has been previously reported (2).

Various categories of roots described include main, secondary, tertiary, and quaternary. Main roots are those which arise directly from the base of the plant. This term is used so that it is unnecessary to distinguish between primary roots of seminal origin and those of adventitious origin which arise directly from the base. Secondary roots arise directly from main roots, tertiary roots from secondaries, and quaternary roots from tertiaries.

Results

Table 1 presents the total number and lengths of roots in each of the various categories, giving both the average individual root length and, in parentheses, the total length for all roots in each category.

In most cases the type of root system was tap. This is shown where the number of main roots is listed as 1 in the first column. In others, as clover, plantain, and the grasses, the root systems were fibrous and therefore had more numerous main roots.

The total number of roots in any category is based upon the actual count of all roots in that category for at least ten plants, and/or upon calculations derived by counting the number of branch roots in a centimeter of root length and multiplying that number by the average length of the category from which the branch roots arise.

Discussion

Roots are not listed in the quaternary division for a number of species, since roots of the tertiary category in these plants did not give rise to laterals. *Setaria viridis* is the only species examined which branched only into the secondary order; there were no tertiaries on plants of this species. In only one plant, *U. pumila*, were there root branches beyond the quaternary division, but these were not included in this study. On the 4-6-year-old elm trees examined, divisions occurred out to the sixth order.

Degree of branching of roots from one category to another may be conditioned by the inheritable stability within the species, age of the plant, and/or extent of root growth as limited by external factors. The writer has noted (2, 3, 4) that winter rye grown well apart from other plants will branch freely into the quaternary division but when grown normally in the field with other rye plants will branch only into the tertiary division. Top development is similarly affected by competition.

The number of secondary roots arising from main roots is no indication of the number of tertiary roots which arise from secondaries. The number of laterals arising from roots, per centimeter, in one category, will be different from the number arising from roots of another category on the same plant. On some plants, as *Salsola pestifer*, the number of tertiary roots greatly exceeded those of the secondary and quaternary divisions. Only about one-fifth of the tertiary roots had one or more quaternary roots arising from them. This was correlated with the fact that on *S. pestifer* the tertiary roots averaged only 2 mm. in length and that roots of this size rarely show any branch-

TABLE 1
NUMBER OF ROOTS AND AVERAGE ROOT LENGTHS FOR VARIOUS SPECIES IN
DIFFERENT ROOT CATEGORIES

Figures in parentheses express total length for all roots in that category

FAMILY AND SPECIES	No. of roots				Average length of roots (cm.)			
	Main	Second-ary	Ter-tiary	Quater-nary	Main	Second-ary	Ter-tiary	Quaternary
Urticaceae:								
<i>Ulmus pumila</i> L.....	I	55	1100	88,000	55	40 (2200)	8.0 (8800)	2.0 (176,000)
Chenopodiaceae:								
<i>Salsola pestifer</i> A. Nels.....	I	105	3150	630	35	10 (1050)	0.2 (630)	0.1 (63)
Amaranthaceae:								
<i>Amaranthus torreyi</i> (Gray) Benth.....	I	40	325	254	8	18.2 (728)	0.8 (280)	0.1 (25.4)
Caryophyllaceae:								
<i>Cerastium arvense</i> L.....	I	14	105	7	2.5 (35)	0.15 (15.7)
Cruciferae:								
<i>Descurainia pinnata</i> (Walt.) Britt.....	I	10	520	1560	5	13 (130)	2.0 (1040)	0.3 (468)
Leguminosae:								
<i>Soya max</i> var. Illini.....	I	51	470	260	11	5 (255)	0.8 (376)	0.4 (104)
<i>Trifolium repens</i> L.....	35	980	137	7 (245)	0.7 (686)	0.2 (27.4)
<i>Pueraria thunbergiana</i> Benth....	I	54	756	9	7 (378)	0.3 (226.8)
<i>Parosela dalea</i> (L.) Britt.....	I	52	1560	1560	13	10 (520)	1.0 (1560)	0.2 (312)
Zygophyllaceae:								
<i>Tribulus terrestris</i> L.....	I	200	160	25	0.8 (160)	0.2 (32)
Euphorbiaceae:								
<i>Euphorbia albomarginata</i> Torr. and Gray.....	I	22	220	11	10 (220)	0.7 (154)
Convolvulaceae:								
<i>Convolvulus arvensis</i> L.....	I	28	168	14	12 (336)	0.25 (32)
Labiatae:								
<i>Nepeta cataria</i> L.....	I	100	1200	200	10	3 (300)	0.5 (600)	0.1 (20)
Solanaceae:								
<i>Solanum eleagnifolium</i> Cav....	I	130	650	65	5 (650)	0.5 (325)
Plantaginaceae:								
<i>Plantago major</i> L.....	26	637	1449	10.5 (273)	1.8 (1147)	0.3 (435)
Compositae:								
<i>Tagetes patula</i> A. Gray.....	I	72	432	12	1.5 (108)	0.3 (130)
<i>Taraxacum officinale</i> Weber...	I	180	900	30	4 (720)	0.4 (360)
Gramineae:								
<i>Setaria viridis</i> (L.) Beauv.....	12	3360	None	14 (168)	0.12 (403)
<i>Cynodon dactylon</i> (L.) Pers....	4 per node	660	22	15 (60)	3 (1980)	0.4 (8.8)

ing. *Descurainia pinnata* had about three quaternary roots arising from each tertiary root, but the length of the latter in this species averaged 2 cm.

In the three species in which tertiary roots averaged 1 cm. or more in length, the number of quaternary roots equaled or exceeded the number of tertiaries. If tertiaries averaged shorter than 1 cm., their number was always greater than the quaternaries. This statement is fortified by the data on *Parosela dalea*, in which the tertiary roots averaged 1 cm. in length, and the number of quaternaries was the same as tertiaries. Other plants listed in the table bear out this conclusion.

The secondary roots, although not so many in total number as tertiaries on most plants because of the shortness of the main root from which they arose, nevertheless were more numerous per centimeter of main root length than were tertiaries per centimeter of secondary root. The number of secondaries per centimeter of main root averaged about five; the smallest number was one per centimeter in *U. pumila*; the largest was twenty per centimeter in *Setaria viridis*. It is interesting that the latter species has no tertiary roots. There is probably a correlation between the facts that secondary roots are more numerous per centimeter of parent root than are tertiaries and that the main root is larger in diameter than the secondaries. The rule does not necessarily follow that a root large in diameter will have many laterals, but main roots are always several to many times larger in diameter than secondary roots of the same plant and invariably have more laterals per centimeter.

Figures by DEAN (1) in her study of aquatic plants, including *Typha latifolia*, show as many as fifty to sixty laterals produced per centimeter of root length in aerated clay soils and five to thirty per centimeter in unaerated clay. WEAVER and HIMMEL (7) counted six to twenty-five laterals per centimeter on the main roots of *T. latifolia* in a similar study. The number of laterals reported in the present paper follow the minimum figures of these other workers. The author, however, has investigated plants under semiarid conditions; these other investigators used aquatic species.

Summary

1. Quantitative studies of the roots of nineteen species of plants in fourteen different angiosperm families were made. Results indicating the total number of roots and root lengths are presented in tabular form.

2. Tertiary roots produced about one quaternary root per centimeter of length in most of the plants examined; however, the number of secondary and tertiary roots developing from main and secondaries, respectively, varied considerably.

3. Variation in number of roots and root length results from differences in size of plant, age, inheritable stability, and competition with other plants. Generally, plants show a compensating factor in root growth; when restricted in one category, another root division or two will exhibit much greater growth.

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GYMNODINIUM BREVIS SP. NOV., A CAUSE OF DISCOLORED WATER
AND ANIMAL MORTALITY IN THE GULF OF MEXICO¹

CHARLES C. DAVIS

GUNTER *et al.* (2) reported a mass mortality of marine animals on the lower West Coast of Florida which was associated with a yellow or yellow-green discoloration of the water and with the occurrence of large numbers of individuals of a species of the genus *Gymnodinium*. In connection with their study of the mortality several samples of the plankton were taken, and, in addition, preliminary study of living specimens of *Gymnodinium* was attempted under difficult field conditions.

In April, 1947, there were further reports of fish mortality in the Gulf of Mexico off the Florida Keys, and on April 12 a field study was made of the situation. Again, associated with mortality, there was a yellowish-green discoloration of the water and the presence of the same species of *Gymnodinium*. Living specimens were more carefully studied, some being returned to the laboratory alive. New outbursts of mortality occurred in July from Venice to

Sarasota. These were also investigated in the field.

This organism was enormously abundant. Off Key West (near Content Keys) fish mortality was occurring in a situation in which the count of *Gymnodinium* was 420,000 cells per liter. At one location near Fort Myers, Florida, during the January mortality, it was 13,900,000 cells per liter and was probably even higher in other localities at that time. In July, off Venice, a sample was obtained containing over 60,000,000 cells per liter. Furthermore, the mortality of fish and other animals occurred sporadically over a period of 9 months from November, 1946, to July, 1947. This very abundant organism appears, however, to be new to science.

Gymnodinium brevis sp. nov.

DIAGNOSIS.—Cella quadrata, 25.3 ad 31.6 μ longa atque lata, 12.7 μ crassa. Zona non disposita. Epicono paulo minore quam hypocono. Hypocono sulco alte denticulato, sulco praeter zonam multum extendente. Epicono magnum

¹ Contribution no. 17, University of Miami Marine Laboratory.

independentem apicalem processum habente. Cella sine maculis exterioribus. Chromatophoros croceo-virides et granula sine colore habente.

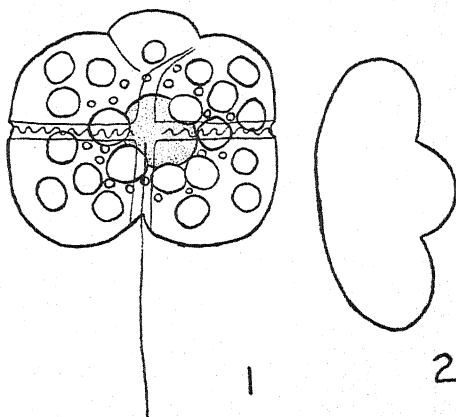
DESCRIPTION.—The body of the living cells is approximately the same in length as in breadth, these dimensions varying from $25.3\ \mu$ to $31.6\ \mu$. The cell, however, is very flat, being only $12.7\ \mu$ in thickness in those living specimens on which this measurement could be made. It is more or less square in outline, with the girdle placed a little closer to the epicone than to the hypocone. There is no displacement of the girdle.

The hypocone is very deeply notched by the sulcus, which itself is deeply impressed. The sulcus extends far beyond the girdle into the epicone, wherein it bends rather sharply to the right to pass around a prominent domelike overhanging process at the apex of the epicone. The sulcus ends just short of the epicone end of the cell. On the left side of the overhanging process, setting it off clearly from the rest of the cell, is a deep impression.

The girdle and the sulcus divide the body of the cell into quadrants. The two quadrants in the epicone are somewhat smaller than those in the hypocone. The domelike process at the apex forms a fifth division, much smaller than the other four. There are no markings on the surface of the body.

The cells contain several chromatophores, which are yellow-green in color in both living and preserved specimens. All color is confined to the chromatophores. Numerous colorless starchlike bodies which, however, do not stain with iodine, occur toward the center of the cell. No sign of ingested food materials was observed.

COMPARISONS.—Because this species has a thin periplast without surface markings, and contains chromatophores, it belongs to the subgenus *Gymnodinium*. It has an overhanging epicone similar to that of *G. scopulosum* as described by KOFOID and SWEZY (3) but cannot be this species because of its general body shape, the presence of chromatophores, differing coloration, lack of displacement



FIGS. 1-2.—*Gymnodinium brevis* sp. nov. Fig. 1, sulcus view. Fig. 2, apical view of epicone to show overhanging process. $\times 970$.

of the girdle, etc. In size, general body outline, and presence of yellow chromatophores, *G. brevis* is similar to *G. agile* Kofoid and Swezy, but the epicone of *G. agile* does not form so large an overhang, its chromatophores are fewer, its cell is not so flattened, and its sulcus does not extend beyond the girdle. In general body form *G. brevis* is somewhat similar to *Phyllodinium scutellaris* as described by CONRAD (1), but *P. scutellaris* does not have an overhanging process, and its sulcus is on the convex surface of the cell, rather than the concave surface.

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CURRENT LITERATURE

Hormones and Horticulture. By GEORGE S. AVERY, JR., and ELIZABETH B. JOHNSON, with the collaboration of RUTH M. ADDOMS and BETTY F. THOMSON. New York: McGraw-Hill Book Co., 1947. Pp. xii+260+51 figs.+26 tables. \$4.50.

Within the last 15 years a group of organic compounds sometimes designated as plant hormones has come into wide economic use in the field of agriculture. One of the substances first used was indole-acetic acid. As time has gone along, other chemicals have been experimented with and have come into use. It is principally these chemicals that the authors refer to in the present book. Other investigators have preferred the use of other terms for them, such as "auxin," "growth-regulator," "growth-regulating substance," "growth-promoting compound," and the like. None of these terms, which imply excitation of growth, is adequate. Many of the chemicals may actually influence growth very slightly, although they may profoundly affect certain physiological processes both qualitatively and quantitatively, either increasing or decreasing their rate. Some of these processes have been shown to be the digestion of starches, proteins, and other substances, uptake or retention of water and minerals, respiration, and the more generalized and complex set of phenomena referred to as enzymatic activity.

Thus the term "hormone" when used in the sense of "arousing to activity" is too limited in meaning to be wholly appropriate or adequately descriptive for these compounds. The word "ergocrine" could be used to indicate the fact that to a degree they more or less precisely affect or regulate what a thing does, for plant responses to them are apparently not haphazard. It may well be, however, that it would be best to await the results from further studies on processes and responses of plants to this general class of compounds before encumbering literature with additional terminology.

The authors point out some of the many applications of "plant hormones" which have been made in the field of horticulture. The uses of these chemicals apply with equal force to the whole field of agriculture in its broadest sense. Still, to say that the use of these compounds in the field of agriculture is revolutionary is somewhat overenthusiastic. Their final economic value has by no means been assessed as yet.

In addition to a brief historical survey, there are chapters dealing with the rooting of cuttings, control of preharvest drop of fruits, the setting of fruits with or without pollination, treating of seeds, weed control, prolonging and inducing dormancy, and miscellaneous growth phenomena. There are also chapters on the use of certain chemicals not regarded as hormones by the authors, in thinning of blossoms on trees, breaking dormancy, and the production of new varieties.

The illustrations are numerous, clear cut, and meaningful. There are a number of tables and lists of plants, presenting a wide array of experimental data. The data given are probably as critical as many of the original, fragmentary reports permit. But many such reported results must certainly be regarded as tentative, pending further experiments under a greater variety of conditions and environments. Even though some of the evidence is fragmentary and conflicting, it is well to have summaries presented from time to time. There is no more reason to assume that the uses of "hormones" in agricultural practice will be wholly standardized and stabilized in the immediate future than have been the uses of mineral and organic fertilizers. Progress is being made and should be reported. Eventually clearer patterns will emerge. It would have been well if greater emphasis had been given the fact that great economic losses may result from improper use of these chemicals.

Each chapter has a summation of much of the pertinent literature referring to the subject matter it treats. There are author and subject indexes. As a whole, the book is very valuable in bringing together some of the many contributions made in this field to date. The enormous rate at which new and additional data are now accumulating—scores of articles every year—makes it impossible for any book to be more than a report of progress. The present book reports well.—E. J. KRAUS.

North American Species of Mycena. By ALEXANDER H. SMITH. Ann Arbor, Mich.: University of Michigan Press, 1947. Pp. xviii+521+99 pls. +56 figs. \$6.00.

Mycena is a genus of small- to medium-sized gill fungi characteristic of temperate forested regions,

including mountain forests in the tropics. In the work here noted 232 species and varieties are described in detail, the great majority illustrated by photographs or drawings of microscopic characters or both. These are practically all from the United States and Canada. In addition, a number of Mexican and West Indian species originally described by MURRILL have been restudied and redescribed with particular attention to microscopic features. Twenty-eight species and seven varieties are described as new, and there are a number of new combinations and new names. Names applying to excluded and doubtful species are listed and critical notes are presented on those of which SMITH has been able to examine authentic material. One new combination is proposed in *Hygrophorus*. The halftones are nearly all excellent and the text figures clear, although unfortunately magnifications are not stated.

As the author points out, much remains to be learned of the genus as it occurs in North America, but the net result of his work is to alter the status of *Mycena* from one of the most difficult and least known to one of the best understood of the North American genera of agarics.—G. W. MARTIN.

Différenciation des tubes criblés chez les Angiospermes: Recherches cytologiques. By JANINE SALMON. 1946. (Copyright 1947 by J. Salmon. Imprimé Oberthur, Rennes, May 1947.) Pp. 235+122 figs.+19 pls.+2 photomicrographs.

This monograph is a Doctor's dissertation which was commenced under the direction of A. GUILLIERMOND. The author also acknowledges the guidance of G. MANGENOT and R. J. GAUTHERET. The topics considered are as follows: nuclear degeneration; cytoplasmic differentiation at the level of the sieve plates; the plasmodesmata; various inclusions of the sieve tubes; chondriome and the question of "red starch"; vacuolar system of the sieve tubes; and general survey of differentiation of sieve tubes and companion cells, primary and secondary phloem. The studies were made on seventy-seven species of sixty-seven genera in forty-five families of dicotyledons and on seven species of as many genera in three families of monocotyledons. Vital staining and various microchemical tests were performed with part of the material; the other was processed through paraffin.

The presentation of original data is preceded by a chronological review of the literature on the structure of the sieve tube. One of the main conclusions drawn by Miss SALMON from this review is that callose and the slimy accumulations on the sieve plate are frequently confused in the literature. This is a surprising conclusion, because even the earliest students of the sieve tube had a good understanding of the distribution of callose on the sieve plate and were able to differentiate between the contents of the

sieve tubes and callose (see review by ESAU, Bot. Rev. 5: 373-432. 1939). In keeping with her misconception regarding the treatment of the subject of callose in the literature, SALMON failed to indicate the substance in certain diagrams (p. 18, fig. 3) which are supposed to summarize the various authors' interpretation of sieve-plate structure. To complete the consideration of her views on callose, as expressed in the main part of the paper, it will suffice to point out that she did not recognize the presence of this substance during the early stages of sieve-plate differentiation (p. 189), reported only on the definitive callus (p. 85-89), and suggested a reversible transformation of the "phospholipoidal material" (slime) into callose (p. 194).

SALMON reports the loss of nucleus in differentiating sieve tubes in fifty-two species of forty-seven genera of dicotyledons and two species in two genera of monocotyledons. An attempt is made to distinguish between two types of nuclear disintegration. One, called "pyncotic," is said to involve an increase in density and chromaticity followed by a dispersal of the nucleus; the other, the "dechromatization," leads to a decrease in density and eventual loss of chromatic contents. Often the two types are not clearly separated and may be combined in the same kind of sieve tube. The various aspects of the disintegrating nuclei are interpreted as distinct phases, are given names, and are illustrated by numerous plates of colored drawings. To what extent fixation may have influenced the appearance of the disintegrating nuclei is not considered. With the progress of the discourse the distinction between pyncosis and dechromatization becomes more and more real in the author's treatment. We read eventually that primary sieve tubes lose their nuclei by pyncotic degeneration, the secondary by dechromatization. Toward the end of the paper the type of nuclear degeneration is used as a criterion for the identification of primary and secondary sieve tubes. In discussing the matter of distinguishing between the two kinds of sieve tubes, the author reveals a peculiar misconception that "the presence of primary phloem is considered to be exceptional in stems of dicotyledons" (p. 182). However, according to SALMON, *Bryonia* stems have primary sieve tubes, because some of these elements show pyncosis of nuclei. We are also told that the sieve tubes in seedling hypocotyl and root of *Bryonia*, which appear to be primary on anatomical grounds, are in reality secondary because they show dechromatization of nuclei (pp. 172-175). Her logic is difficult to follow when we remember that on page 37 the occurrence of primary sieve tubes in *Bryonia* stems was taken as a matter of course and that presumably the identification of these elements was made on anatomical grounds before their nuclear behavior was known. Despite the detailed study of nuclear degeneration, SALMON failed to note the extrusion of nucleoli in species showing this phenomenon (ESAU, Amer. Jour. Bot. 34: 224-233. 1947).

The part dealing with plasmodesmata of the sieve plates contains even more errors of fact and inference than the pages just considered. SALMON does not seem to be aware of the problems involved in the interpretation of the origin of connecting strands in the sieve plates and simply states: "The plasmodesmata begin to form at the time when the cellulose wall of the sieve plate is pierced" (p. 56). No reference is made to the common idea that plasmodesmata may be present in the walls from the latter's inception. Although the wall is "pierced," the plasmodesmata of the two superposed elements do not fuse, according to SALMON. A discontinuity remains between the plasma membranes of the two opposed plasmodesmata. Special terms are used to characterize the situation. Following MANGENOT, SALMON speaks of a synopsis of membranes, and, probably because these membranes readily stain with iron hematoxylin, they are referred to as siderophilic membranes. Thus each sieve-plate pore is said to contain a couple of siderophilic membranes. Although the author admits that the discontinuity of the plasmodesmata is not always visible, she nevertheless assumes, on scanty evidence, that bounding membranes are always present.

The plasmodesmata later undergo a modification associated with the appearance of the darkly staining material on the sieve plate (which is spoken of as "slime" in the literature written in English). SALMON interprets this material as a plasma membrane of the sieve-tube protoplast and finds that it is proteinaceous and phospholipoidal in nature. At first the plasma membrane is thin; later it thickens asymmetrically: becomes thicker on the "lower" sieve plate. This asymmetry is said to be a reflection of the polarity of movement in the sieve tubes. Thus the well-known "slime plugs," which appear as artifacts on the sieve plates when the phloem is cut, are considered to be normal structures, namely, plasma membranes.

The pulling-away of the sieve-tube protoplast from the longitudinal walls, which the author brings into relation with the thickening of the "plasma membrane," is described as a normal phenomenon also. We are told that, because of this contraction of cytoplasm in mature sieve tubes, plasmolytic tests are not applicable to them (pp. 199-200). Though the sieve-tube slime is interpreted as a plasma membrane, it is referred to as "accumulations cribrales" in most of the subsequent discussion. In the part dealing with the slime bodies SALMON states that these are proteinaceous and phospholipoidal in nature ("corpuscules lipido-protéiques") and that their substance becomes incorporated in the accumulations on the sieve plates.

The young sieve tubes have osmiophilic inclusions, which are interpreted as microsomes. They

also take part in the formation of the "accumulations cribrales." The sieve-tube plastids are derived from mitochondria, and the material that is elaborated in these plastids and stains red with iodine is described as a juvenile form of true starch, which is degraded by amylase but is nonrefractive. The early breakdown of the plastids prevents the development of the grains into ordinary starch. In a recapitulation, she generalizes that in dicotyledons each plastid elaborates several starch grains and, in the monocotyledons, starch grains and lipoidal granules. By means of neutral red the growth of vacuoles in number and size and their fusion into one vacuole were studied. The lack of accumulation of this stain by differentiated sieve tubes is also mentioned.

One more unusual feature is reported by SALMON. The companion cells of *Cucurbita*, *Bryonia*, *Urtica*, and *Robinia* are said to differentiate into sieve-tube elements after the associated sieve tubes had been fully formed. The evidence is presented by means of drawings in which the end walls of the companion-cell series often do not agree with the end walls of the associated sieve-tube elements. This peculiarity is explained as resulting from a displacement of their walls during the subsequent growth of the companion cells. The fact that ordinarily the companion cells of one element do not occur in a continuous file with those of another, superposed element is not considered. There is also a reference to a progressive shift in the relation between the sieve-tube element itself and one of its inclusions, if different stem levels are compared. Toward the apex, successively less differentiated cells—that is, shorter cells with less and less distinct sieve plates—show the appearance of slime bodies. The fact that at higher levels the differentiating sieve tubes could be the first proto-phloem sieve tubes—and those farther down, the corresponding cells of the metaphloem, or even secondary phloem—is ignored.

In conclusion it might be proper to list the observations of SALMON which, in the opinion of the reviewer, could be regarded as contributions to our knowledge of the characteristics of sieve tubes. Here, one could name, first, the evidence that so many different dicotyledons and some monocotyledons show the common phenomenon of nuclear disintegration in differentiating sieve tubes; second, the record that slime bodies have been observed in a wide variety of dicotyledonous plants. Perhaps the identification of lipids in the sieve-tube slime may be mentioned also, if the accuracy in the execution of the microchemical tests can be relied upon. All the other conclusions are inadequately supported by evidence, and some directly contradict the well-known facts of plant structure and differentiation.—KATHERINE ESAU, College of Agriculture, University of California, Davis, California.

REPRODUCTIVE STRUCTURES OF THE GAMETOPHYTES OF
HYMENOPHYLLUM AND TRICHOMANES

ALMA G. STOKEY

Introduction

The first account of the reproductive organs of the gametophytes of the Hymenophyllaceae was given in 1864 by METTENIUS (17). He had examined the tangled material at the bases of leafy plants in herbarium specimens of *Trichomanes incisum*, *T. sinuosum*, and an undetermined species and had found gametophytes bearing sex organs and gemmae. JANCZEWSKI and ROSTAFINSKI (16), in 1875, described the mature gametophytes of *Hymenophyllum tunbridgense* from specimens collected near Cherbourg; this account includes the external aspect of the sex organs with a few details of their structure obtained from hand sections, but no figures were given. GOEBEL (11) in 1887 gave descriptions and figures of the external aspect of the sex organs of *H. eximium*, *H. dilatatum*, *H. smithii*, *T. digitatum*, and *T. diffusum*; and in 1892 (12) he described the gametophytes of *T. rigidum* and *T. sinuosum*. In 1888 BOWER (1, 2) published accounts of gemmae formation and apogamy from his investigation of *T. pyxidiferum* L. and *T. alatum*; and in 1894 he continued with an account (3) of apospory and gemmae formation in *T. kaulfussii* Hk. and Grev. GEORGEVITCH (8, 9) further extended our knowledge of *T. kaulfussii* with accounts of apogamy, apospory, and antheridial structure. HOLLOWAY (14) reported on sex organs in field collections of *H. pulcherrimum* Col., although none had appeared in cultures 3½ years old. In 1944 he gave an account (15) of the sex organs and embryo of *Cardiomanes reniforme*

(Forst.) Pr. based on gametophytes in culture for over 6 years. This is the only account of the internal structure of the archegonium of any member of the Hymenophyllaceae except for the limited descriptions by JANCZEWSKI and ROSTAFINSKI.

Material and methods

The material used in this study was from sets of cultures described in an earlier paper (18) in which an account was given of spore germination and vegetative stages of seven species of *Hymenophyllum* and four of *Trichomanes*. Of these, four species of *Hymenophyllum* and three of *Trichomanes* lived long enough to bear antheridia, and with the exception of one species of *Trichomanes* all bore archegonia. The culture of *H. blumeanum* Spr. was started in Formosa in 1931, and the others in Java in 1937. They have been transplanted at intervals ranging from 6 months to 3 years. Four species are still in culture. A fuller account of the cultures as well as the author's many obligations for material, assistance, and laboratory facilities are given in the earlier paper.

The external aspect of the sex organs and their distribution were studied in fresh material. The surface view of the antheridium of *Hymenophyllum* was also examined in material bleached in 4% potassium hydroxide and stained in Mayer's haem-alum. The antheridium of *Trichomanes* is usually so much smaller than that of *Hymenophyllum* that its external aspect was usually clear in fresh material. Microtome sections were used

for the study of internal stages of development. Small portions of the ribbon-like thallus of *Hymenophyllum* bearing sex organs were cut out, and portions of the filamentous gametophytes of *Trichomanes* bearing archegoniophores were carefully dissected out to obtain material for paraffin sections. This material was treated with various killing and fixing agents; the most satisfactory was Navashin's fluid (Craf formula) in two-thirds or three-fourths strength. Flemming's triple stain gave the best results of the various stains tried. Fertile portions in large numbers were imbedded in paraffin and cut in mass at $10\ \mu$, since the archegonium-bearing cushions of *Hymenophyllum* and the archegoniophores of *Trichomanes* are too small and unsymmetrical for the orientation of sex organs or regions. Sufficiently large amounts of fruiting material, except for antheridia of *Trichomanes*, were available to afford an adequate number of sections for the interpretations of the structures.

Investigation

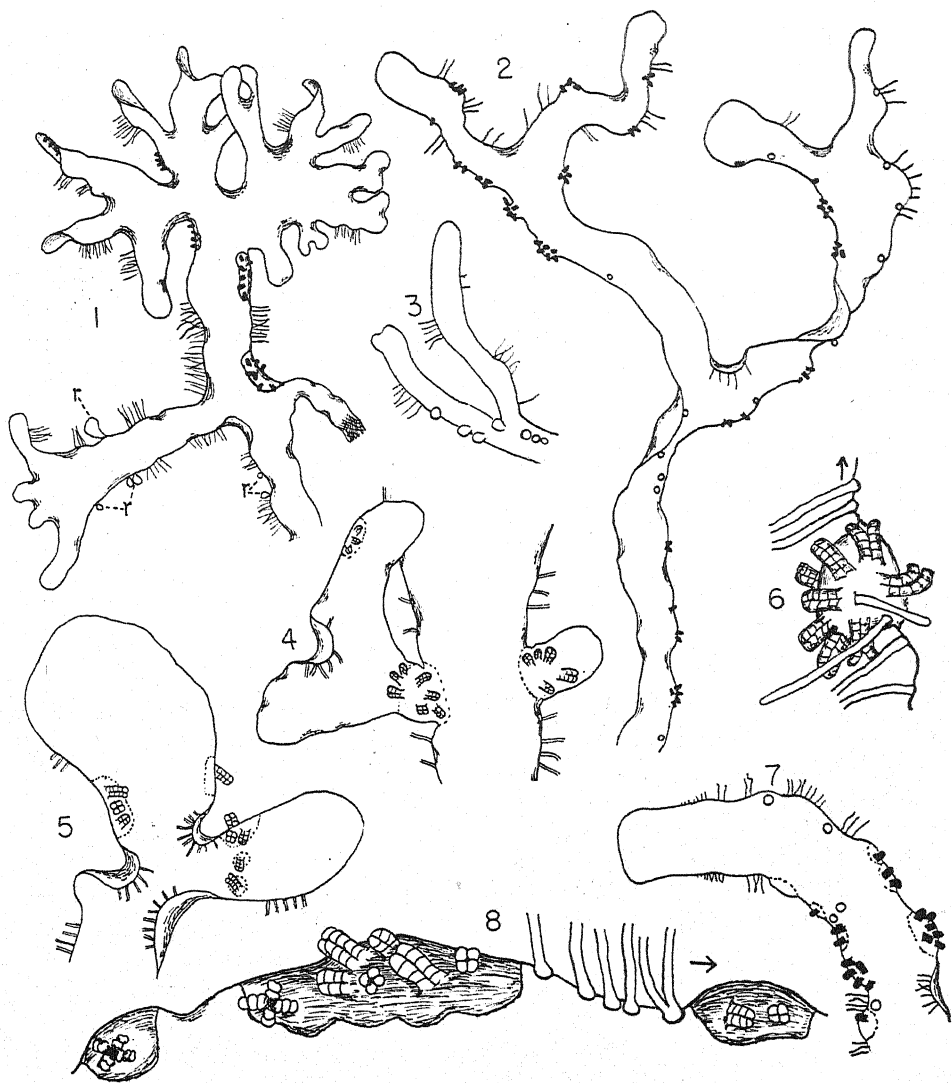
The gametophytes of the Hymenophyllaceae are relatively slow in development, but they seem able to live in culture almost indefinitely even with considerable neglect. *Hymenophyllum blumeum* Spr. was in culture over 6 years; archegonia were not observed until the cultures were more than $3\frac{1}{2}$ years old, and no antheridia were ever seen (fig. 1). Since it was not anticipated that the prothalli might be dioecious, no attempt was made in this first culture of *Hymenophyllum* to select portions from various parts of the tangled mass of branches when making transfers. When archegonia were first observed, they were found not only near the tips of the gametophyte branches but farther back, and they may have arisen perhaps a year earlier, escap-

ing notice because of their scarcity in the culture. Although archegonia appeared at intervals for over 3 years, diligent search revealed no antheridia. The gametophytes of *H. holochilum* (v.d.B.) Chr. and of *H. kurzii* Prantl bore antheridia when about 20 months old, and shortly afterward archegonia were found on the same gametophytes. *H. acanthoides* Ros. did not bear antheridia until 3 years old; it was nearly a year later before archegonia were found, but they were never on the same gametophyte. *T. auriculatum* Bl. and *T. bilabiatum* Nees and Bl. bore antheridia when 9 months old and archegonia about a month later. *T. maximum* Bl., which was exceedingly slow in vegetative development, bore antheridia when a year old but died about 3 months later without having borne archegonia.

HYMENOPHYLLUM

Sex organs developed mostly during the summer in Massachusetts, and no archegonial cushions were formed in winter. The cultures were growing at a north window which subjected them to a lower temperature in winter, during which time the light was weaker and of shorter daily duration. When cultures were examined with a hand lens, the archegonial cushions could be detected by the paler color of the marginal region. The antheridia were less easily detected without dissection.

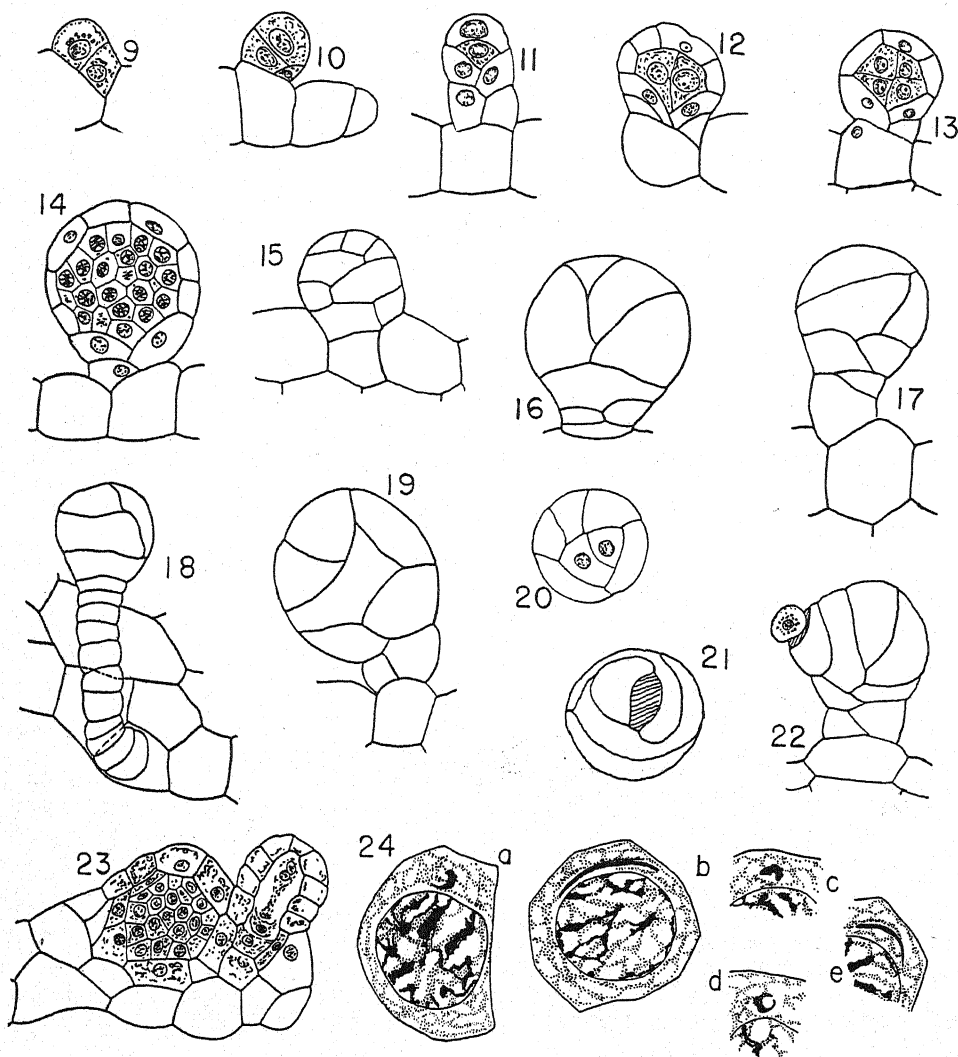
ANTHERIDIA.—The antheridia were found most frequently arising from cells on or near the margin on the ventral side of the ribbon-shaped thallus, less frequently on the dorsal side (figs. 2, 3, 7). The mature antheridium is similar to the more or less complicated type found in the Osmundaceae and Gleicheniaceae. The antheridium arises from an initial cell which is separated from the thallus cell by a wall which may be parallel to



FIGS. 1-8.—Fig. 1, *H. blumeianum*, dorsal view of gametophyte bearing archegonia and several regenerated branches, *r*. Fig. 2, *H. kurzii*, ventral view of gametophyte with antheridia (circles) and archegonia (shaded black). Figs. 3-5, *H. acanthoides*: fig. 3, branch with antheridia; figs. 4, 5, ventral view of branches with archegonia. Figs. 6, 7, *H. holochilum*: fig. 6, cushion with archegonia and rhizoids, ventral side; fig. 7, branch with antheridia and archegonia, ventral side. Fig. 8, *H. kurzii*, margin of gametophyte with three archegonial cushions, ventral view.

the surface but is more frequently oblique (fig. 9). The basal portion of the antheridium is formed by one or, more commonly, two or three wedge-shaped cells (figs. 10, 12); an early stage of the antheridium with the characteristic oblique walls is shown in figure 10. Some-

times a few cells formed by intersecting walls make a short stalk (figs. 11, 17, 19, 22), or occasionally a series of disklike cells lifts the antheridium a considerable distance above the level of the thallus (fig. 18). After several oblique walls have cut off the cells which make up the lateral



FIGS. 9-24.—Figs. 9-15, 20, 21, *H. kurzii*: figs. 9-14, stages in development of antheridium; fig. 15, surface view of antheridium; fig. 20, top view of antheridium almost mature; fig. 21, top view of antheridium after discharge of sperms. Figs. 16-19, 22, *H. acanthoides*, surface views of antheridium. Fig. 23, *H. holochilum*, imbedded antheridium and mature archegonium. Fig. 24, *H. kurzii*: a, b, spermatids with blepharoplast in cytoplasm; c, d, e, stages of blepharoplast.

walls of the antheridium, a cap cell is formed, delimiting the primary spermatogenous cell (fig. 11); early divisions of the latter are shown in figures 12 and 13. The last division of the cap cell, cutting out the opercular cell—which in this case is triangular—is shown in figure 20. The opercular cell is not usually as conspicuous or as regularly placed or formed as in the Osmundaceae or the Gleicheniaceae. It has been stated that there is no opercular cell in *Hymenophyllum* (5), and HOLLOWAY (15) wrote that there is none in *Cardiomanes*. Although the discharge of the opercular cell was not seen, a dislodged cell was found in a number of cases in the vicinity of the opening (fig. 22) or the opening itself was seen (fig. 21). Surface views in figure 15-22 show some of the modifications in the form of the cells of the wall as the antheridium enlarges and matures. The interpretation of the external structure of the antheridium is not easy or certain, since the antheridium is relatively large and since the wall consists of so many cells in which there is considerable variation in both number and form. Some of the larger antheridia show more than thirty sperms in a section. In several preparations of *H. holochilum* partly imbedded antheridia were present (fig. 23). While the cultures were young, the antheridia opened normally and active sperms were seen; for the last 2 or 3 years, however, although antheridia continued to form, none was found which had discharged active sperms.

In the spermatid before the changes occur which result in the formation of the coiled sperm, a dark-staining granular body, undoubtedly the "blepharoplast," is seen in the cytoplasm outside the more or less spherical nucleus (fig. 24, *a, c, d*). In somewhat older cells this body forms a

band, one side of which comes to lie close to the nuclear membrane (fig. 24, *b, e*).

ARCHEGONIA.—The archegonia are borne on more or less erect branches or on branches spreading horizontally or obliquely somewhat above the surface of the substratum. They appear in restricted regions or cushions two cells thick along the margin of the thallus. They were found only on well-developed gametophytes and were more abundant on the peripheral branches of a clump than on those near the center. The young cushions appear not far behind the tip of an actively growing branch. The archegonia usually develop on the ventral side of the thallus—the side away from the light—but occasionally appear on the dorsal side (fig. 5), as was noted by JANCZEWSKI and ROSTAFINSKI in *H. tunbridgensae*. The usual relation of the cushions to the tip of the thallus and the margin is shown in figures 2, 5, 7, and 8, although occasionally a small cushion with one or more archegonia was found in the central portion of the thallus (fig. 5). The cushions vary greatly in size; most of those seen had two to twelve archegonia, but old cushions were found bearing only one archegonium (fig. 8). Even on large vigorous gametophytes only a few branches were found to bear archegonia, and these were branches which had broadened considerably. The slender elongated branches either were sterile or bore antheridia only. When a cushion develops, the margin of the thallus may remain unchanged in form, show a slight modification, or extend into more or less of a lobe (figs. 1, 6), but growth beyond the cushion is rare (fig. 4). The enlargement of the cells on the dorsal side of the cushion may not keep pace with that resulting from the development of archegonia on the ventral side. Then, particularly in the case of

large cushions which have formed a lobe, there may be a curling of the thallus which brings the archegonia into view from the dorsal side (fig. 1). A ventral view of one with that habit is shown in figure 6. In *H. holochilum* there were a few cases in which antheridia were found arising from the archegonial cushion, as was found by GOEBEL (11) in *H. dilatatum* Sw.; but ordinarily they were scattered on the one-layered portion of the thallus. Although, in general, rhizoids are restricted to marginal cells and rarely appear farther back, they are sometimes borne on archegonial cushions (fig. 6).

The archegonium begins its development in the manner typical of leptosporangiate ferns by the division of the initial into an outer cell which forms the primary neck cell and an inner cell (fig. 25). This was observed by JANCZEWSKI and ROSTAFINSKI in *H. tunbridgense*. In the material described in the present paper no cell was recognized with any degree of certainty as the archegonium initial. The inner cell divides into a central cell and a basal cell, making the usual "row of three." At this stage the basal cell may be distinctly larger than the central cell which is made conspicuous by its dense protoplasmic contents rather than by its size (fig. 26). The primary neck cell divides in the usual manner into four cells, each of which gives rise to a row of neck cells (figs. 27, 28). The basal cell may divide horizontally while the archegonium is still very young, cutting off a tabular cell which is a precocious contribution to the jacket layer of the venter (figs. 29-31, 33). In one young archegonium the wall in the basal cell was slightly oblique, making a wedge-shaped cell which did not extend completely across the basal cell (fig. 31).

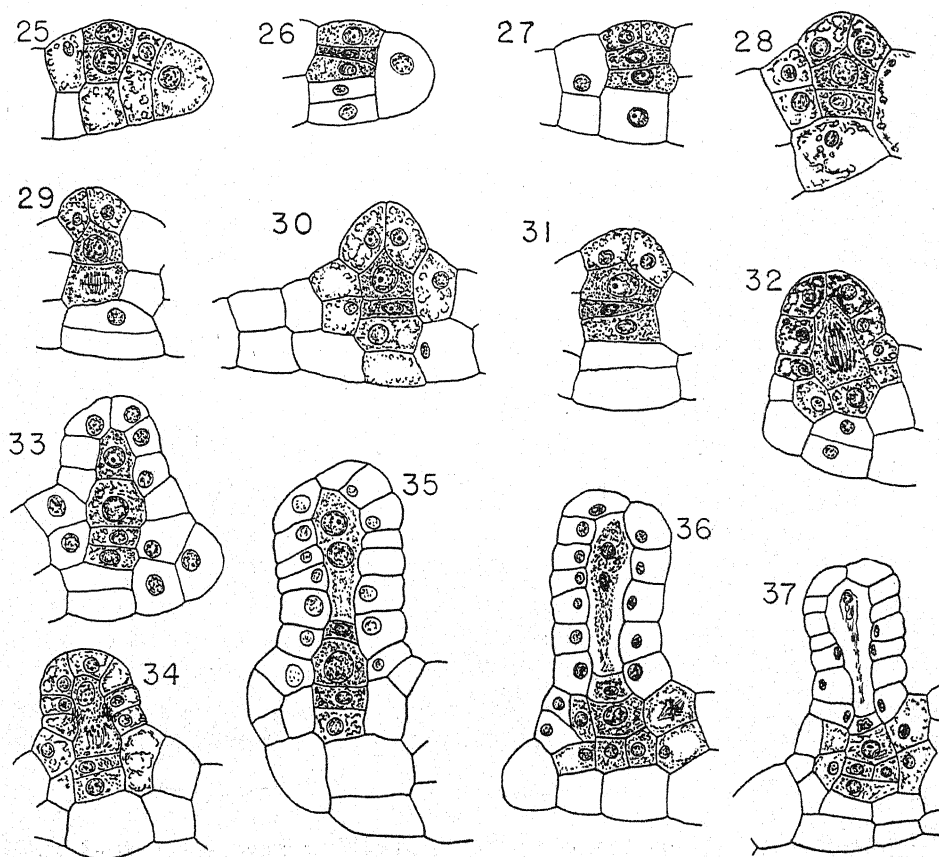
After the separation of the primary neck canal cell and ventral cell, the next

division is that of the central cell to form the egg and ventral canal cell (fig. 34). The neck canal nucleus divides to form two nuclei which may be of considerable size and unusually conspicuous. The nucleus nearer the tip of the neck in figure 35 measured 13.5μ . In no case was a wall found between the neck canal nuclei. There were no archegonia found with an increase in the number of canal nuclei to three or four, as has been found in the Schizaeaceae, Cyatheaceae, and Dicksoniaceae. As the archegonium develops, the neck cells divide until there are six to nine, usually seven, cells in a row. The neck is approximately straight at maturity. The number and size of the cells in the rows may vary, giving a slight tilt to the neck, but the direction of the tilt is not constant. The juncture of the four cells at the tip of an approximately straight neck is often not in the longitudinal axis of the archegonium and suggests a slight inclination to one side (figs. 35-37). The jacket layer of the venter is poorly developed in such stages as those shown in figures 35-37. It is much less conspicuous than in the higher families of leptosporangiate ferns and is more like that of the Marattiaceae. Figures 35-37 show mature archegonia with three types of development of the basal cell: a horizontal division, a vertical, and a horizontal followed by a vertical in the cell adjoining the venter. Because fertilization and development of the embryo were not observed, there was no opportunity to see to what extent the jacket layer develops after fertilization. In the culture of *H. kurzii* fertilization occurred occasionally, and young sporophytes appeared, but no stages of the embryo were found in the material which was imbedded and sectioned.

REGENERATION.—Regeneration occurred frequently under certain condi-

tions in these four species of *Hymenophyllum*. It usually was found on old branches where there was crowding or shading, especially on the branches which became prostrate after having been more or less erect. In figure 1 there are several regenerated branches (γ), recognizable by their slender connections with the old branch. Regeneration usually occurs in regions in which some of the cells are beginning to turn brown and die. The brown areas are indicated by crosshatching in figures 38, 40, and 41. Apparently any marginal cell on an old portion of the thallus is capable of division and may produce regenerated branches for a certain period. Figures 38

and 44 show early stages; figures 39 and 40, slightly later stages. A regenerated branch usually arises from a single marginal cell, but occasionally the products of adjoining cells may combine for a longer or shorter period and later split away (figs. 40, 41). The first wall in the enlarging cell may be horizontal or oblique. The cell at the base may divide vertically or obliquely or remain undivided (figs. 38, 39, 44, 45). The early divisions may arise from a cell with two cutting faces (fig. 39), or the first divisions may be alternately horizontal and vertical (fig. 40) or of a less regular pattern. Although regeneration results in a



FIGS. 25-37.—*H. kurzii*: figs. 25-34, stages in development of archegonium; figs. 35-37, mature archegonia.

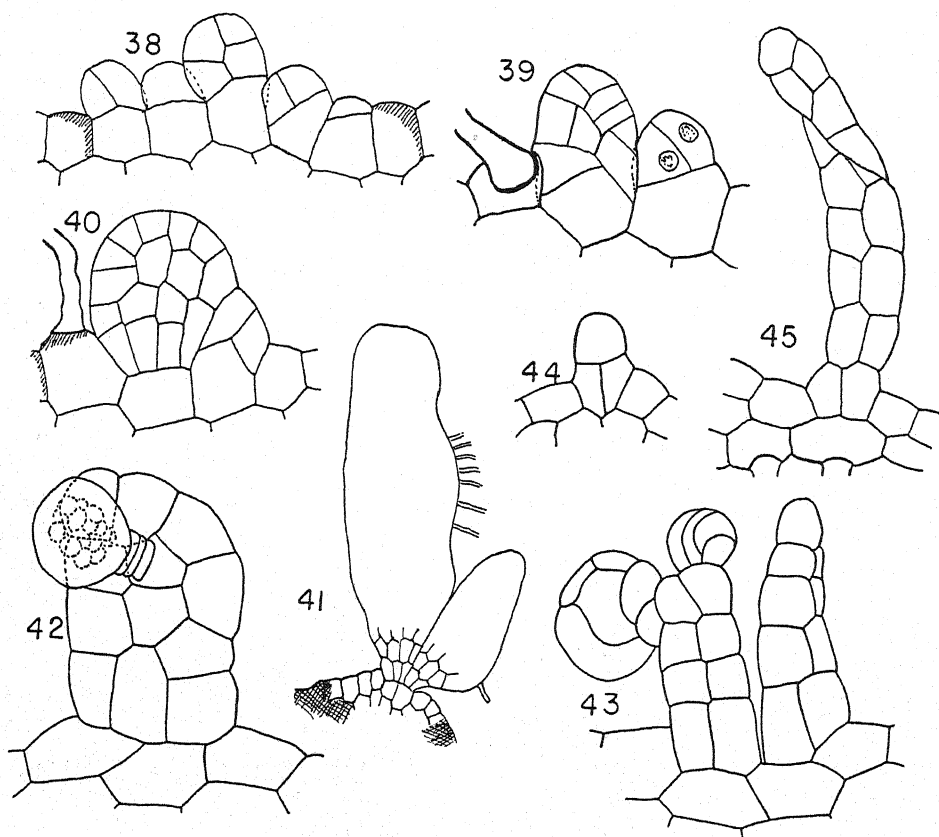
type of branching, it is very unlike that which occurs at the apices. The regenerated branches ultimately fall away from the parent plant and serve as a means of vegetative propagation. The early development of rhizoids, which is helpful in detaching the branches, was not seen in any of these species. GOEBEL (13) has figured it for an undetermined species of *Hymenophyllum*, and it is common in other families of ferns. Regenerated branches were found bearing antheridia while still attached to the parent plant (figs. 42, 43).

None of the species of *Hymenophyllum*

under investigation produced gemmae such as GOEBEL (11) described for *H. eximium* and some undetermined species. CAMPBELL (5) wrote that in an undetermined Hawaiian species of *Hymenophyllum* gemmae occurred abundantly on gametophytes which had ceased to form sex organs.

TRICHOMANES

ANTHERIDIA.—Antheridia appeared much less frequently than archegonia in these cultures of *Trichomanes*, particularly as the cultures aged. The antheridia were much less abundant at any time

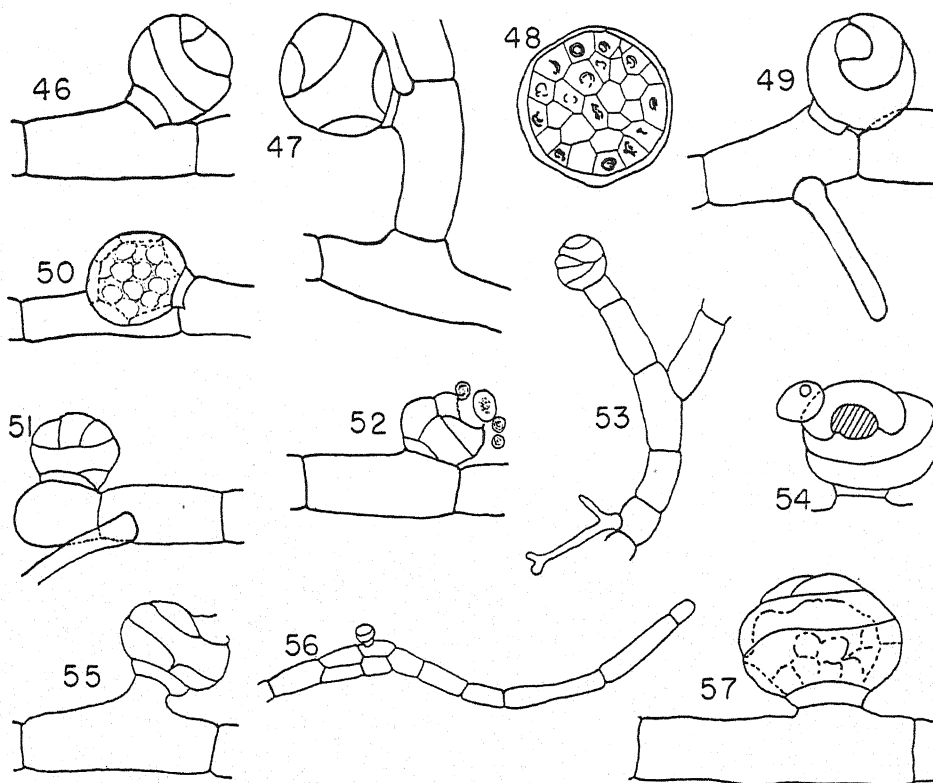


FIGS. 38-45.—Figs. 38-43, *H. acanthoides*: fig. 38, marginal cells in early stages of regeneration, adjoining cells turning brown; fig. 39, two stages in regeneration; figs. 40, 41, regenerated growth initiated by two marginal cells; figs. 42, 43, young regenerated branches with antheridia. Figs. 44, 45, *H. blumeianum*, regenerated branches with vertical division in cell at base.

than on the gametophytes of *H. acanthoides* and *H. kurzii*. They did not have any definite position in relation to the archegonia and were seldom found near them. The two types of sex organs were observed on the same plant only in the case of *T. auriculatum*. This does not necessarily mean that they occur on different plants in *T. bilabiatum*, since in tangled cultures of profusely branching filaments it is difficult to be sure of the relationship of branches detached for examination. The mature antheridium is found on green branches, usually near the anterior end of a cell at some distance behind the tip of a filament; but in some cases, particularly in *T. maximum*, the antheridium is borne on a terminal cell

(figs. 51, 53). A branch may bear a single antheridium or develop them on several successive cells. In only one case was an antheridium found which was not on a uniseriate filament, and that was in a 4-year-old culture of *T. auriculatum* (fig. 56).

The antheridia of the three species of *Trichomanes* are, on the average, smaller than those of the four species of *Hymenophyllum* studied and are simpler in structure. There is a basal cell which is disk-like or wedgelike (figs. 46, 47, 51-53, 57), and occasionally there are two basal cells. The antheridium in its structure suggests those of *Alsophila* rather than those of *Osmunda* or *Gleichenia*. The number of sperms found in a cross section



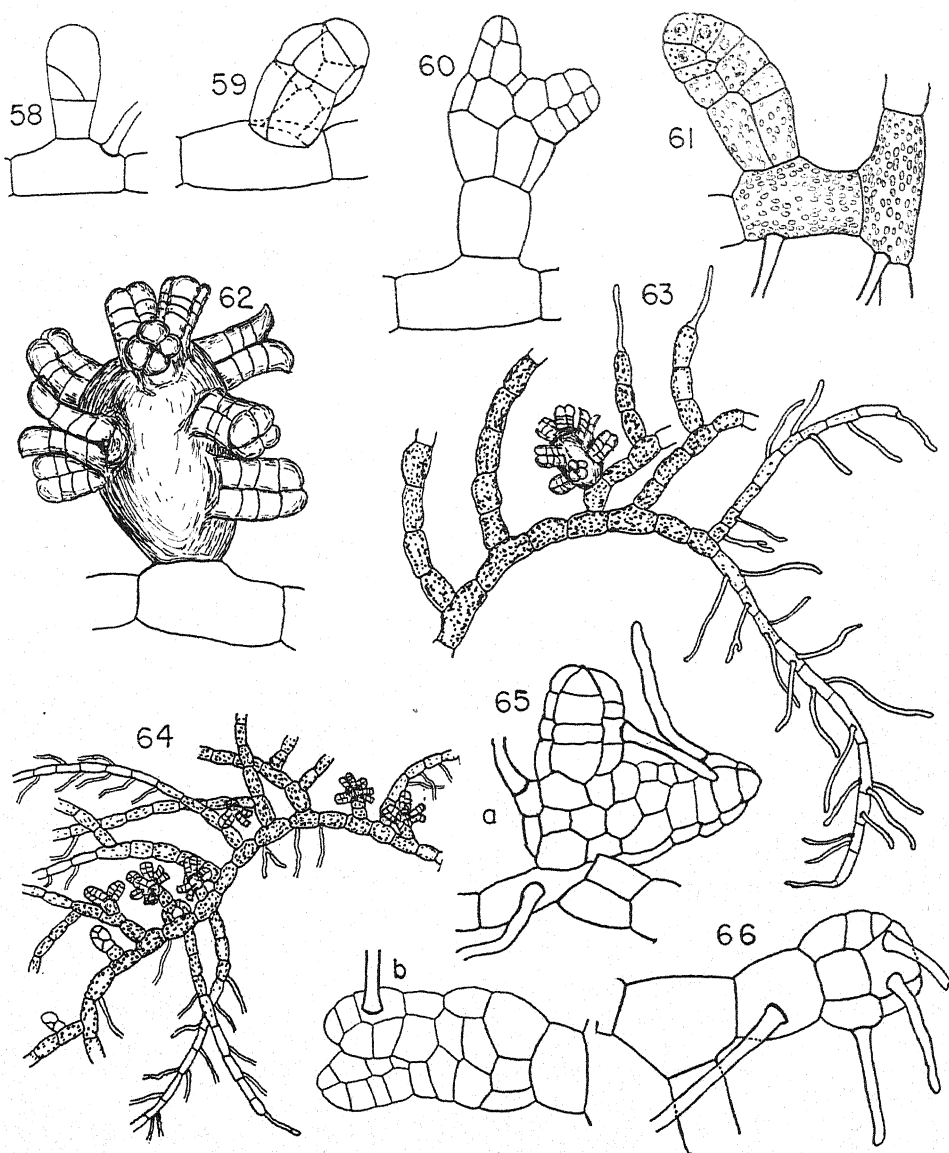
FIGS. 46-57.—Figs. 46-50, *T. bilabiatum*, surface views and sections of antheridium. Figs. 51-53, *T. maximum*, antheridia on filaments. Figs. 54-57, *T. auriculatum*, antheridia on filaments and on expanded branch.

of an antheridium of *T. bilabiatum* is usually between ten and fifteen, but in the larger antheridia of the more vigorous plants it may be as high as twenty-five (figs. 48, 50). The antheridia of *T. auriculatum* are smaller, and very few were seen in which the number of sperms in a cross section was higher than fifteen. The antheridia of *T. maximum* are even smaller, but, since the culture did not live so long as those of the other two species, no opportunity was given for the development of the larger antheridia which may be found on old vigorous gametophytes. The dehiscence of the antheridium is brought about by the throwing-off of the opercular cell (figs. 52, 54). Active sperms were seen in the earlier cultures but, as in *Hymenophyllum*, were not found in cultures more than 7 or 8 years old. Few sections were obtained in the imbedded material because of the scarcity of antheridia in the cultures.

ARCHEGONIA.—These organs arise on specialized archegoniophores which develop as lateral, rarely terminal, outgrowths from the filaments. The archegoniophores arise from green filaments which are always associated with pale or colorless filaments conspicuous because of the abundant development of rhizoids—often two or even three on a cell (figs. 63, 64). The fertile branches were found in the periphery of vigorous clumps and not in the turflike regions in the center. The pale slender branches cling closely to the substratum or penetrate its surface; the transition from green cells to colorless is gradual to abrupt. No archegoniophores were ever found on the expanded bladelike branches of *T. auriculatum*. (No expanded branches have ever developed in *T. bilabiatum*, although it has been in culture for over 10 years.) The initial cell of the archegoniophore arises like that of a

lateral branch but is larger in diameter. There is usually a stalk cell (figs. 58, 60), but the archegoniophore may be sessile on the filament (figs. 59, 61). In either case, divisions by intersecting walls lead to the formation of a globular or ovoid mass. The archegoniophore appears to develop, at least in the earlier stages, as if from an apical cell with three cutting faces. Then periclinal and anticlinal walls are formed, resulting in the formation of a globular, oblong, or irregular mass without any obvious cell pattern (figs. 62, 65). Two archegoniophores may arise from the same basal cell, or an archegoniophore may branch and give rise to a secondary archegoniophore. The cell at the base in figure 67 arose from an older and much larger archegoniophore. The archegoniophores vary greatly in the number of archegonia which they produce. The meristematic activity of the archegoniophore may cease when there are only two or three archegonia, although usually not until there are six or eight, or it may continue until there are over twenty. A large terminal archegoniophore of *T. auriculatum* showed nineteen archegonia in one view. The development of rhizoids on the archegoniophore or even on the archegonia is not unusual (fig. 65). A few cases were found in which growth of the archegoniophore was arrested before archegonia had developed, and on such structures rhizoids were usually present (fig. 66). These must be regarded as arrested archegoniophores and not as vegetative branches, since they were more than two cells thick.

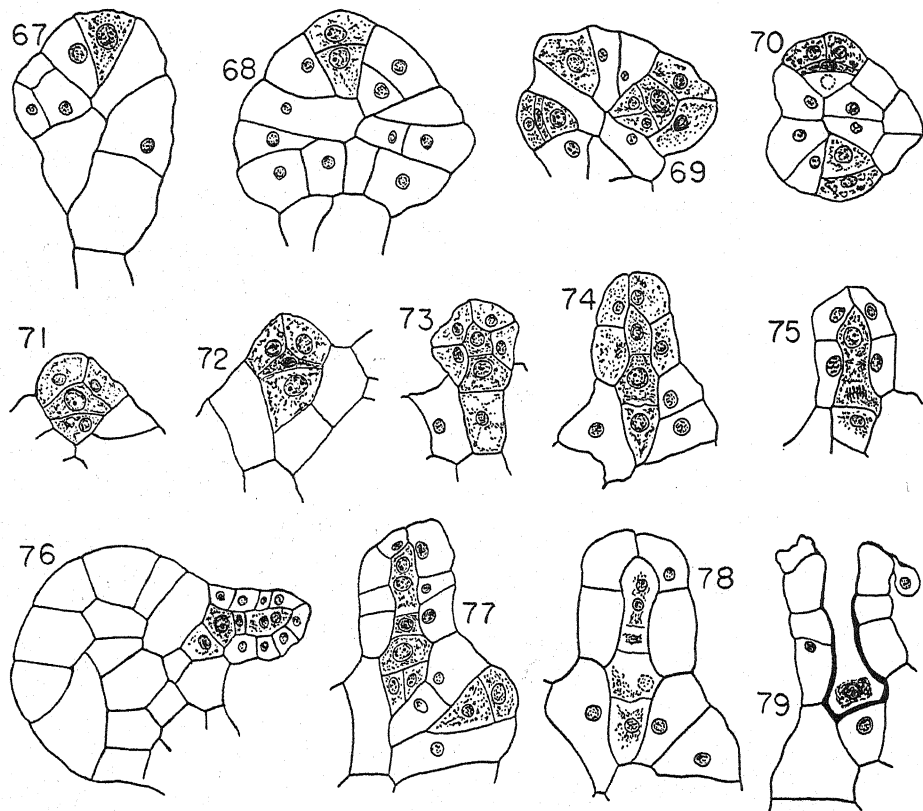
The archegonium initial as seen in section is wedge-shaped (fig. 67). The large, shaded upper cell in figure 69 is probably an initial, although its nucleus differed less strikingly from those in the cells around it than the nucleus in figure 67.



FIGS. 58-66.—Figs. 58, 61-63, 65, *T. bilabiatum*: figs. 58, 61, young archegoniophores; fig. 62, archegoniophore with mature archegonia; fig. 63, green filaments with archegoniophore and colorless rhizoid-bearing filaments; fig. 65, archegoniophores with archegonia and rhizoids. Figs. 59, 60, 64, 66, *T. auriculatum*: figs. 59, 60, young archegoniophores; fig. 64, green filaments with archegoniophores of various ages and pale, rhizoid-bearing filaments; fig. 66, arrested archegoniophore with rhizoids.

The first division gives rise to the primary neck cell (figs. 68, 70, 77). The subsequent horizontal division of the inner cell forms the central cell and the basal cell (fig. 69). As in *Hymenophyllum*, the

larger number than four. In cases in which the number was smaller, it was not certain that the archegonium had completed its development, since spindles may be found in the terminal cells of the



FIGS. 67-79.—Figs. 67-69, 71, 73-79, *T. bilabiatum*: figs. 67-69, 76, longisection of archegoniophore with stages in development of archegonium; figs. 71, 73-75, 77, stages in development of archegonium; fig. 78, aberrant archegonium; fig. 79, old archegonium. Figs. 70, 72, *T. auriculatum*: fig. 70, cross section of archegoniophore with two young stages of archegonium; fig. 72, young archegonium.

growth of the central cell is retarded, and in the early stages the basal cell may be much the larger of the two (figs. 69, 70, 72). The primary neck cell undergoes the usual divisions into four cells (figs. 69-72), and each of these gives rise to a row of four cells (figs. 76-79). The number of cells in the rows of the neck was found to be unusually constant in these two species. No archegonia were found with a

neck when the axial row is complete and the archegonium of almost mature size. The archegonium shown in figure 77 was probably not mature; but in that shown in figure 78 growth had apparently ceased, and the archegonium may be regarded as aberrant. The first division of the central cell separates the neck canal cell from the ventral cell (fig. 73). The next division results in the formation of

the egg and ventral canal cell (fig. 75). As in *Hymenophyllum*, there was no case found in which a wall separated the two neck canal nuclei; there was not even a suggestion of a break in the protoplast. There was no case found of an increase in the number of neck canal nuclei to three or four. As in *Hymenophyllum*, the jacket layer of the venter is not well developed at the time the archegonium is apparently mature or when the neck has opened (figs. 76-79). The basal cell may continue to show the pointed tip seen in the initial and early stages, or it may become more or less obtuse. Its form is determined by the number and position of the walls which impinge on it, and these factors are related to the extent to which cell division and enlargement have taken place in the archegoniophore.

Fertilization was not found in any of these cultures, although sperms were discharged and swam vigorously. Archegonia opened and had the appearance of being receptive, but antheridia were not sufficiently abundant to give a favorable chance for fertilization.

APOGAMY.—Apogamous growths appeared in the cultures of *T. auriculatum* when they were about 4 years old. The simplest type was an expanded blade which bore not only rhizoids, which would be characteristic for such a structure, but also glandular hairs such as are found on the sporophyte (figs. 80, 85). In other cases a leaflike blade with midrib developed; the midrib consisted, in part, of sclerenchymatous cells, and the leaf bore glandular hairs on the wings and midrib (figs. 81-84). The leaves in all these cases were found to bear rhizoids, sometimes filaments, as well as glandular hairs, and the rhizoids arose from the surface as well as from the margin. In some cases the apogamous structure arose from a filament which expanded either

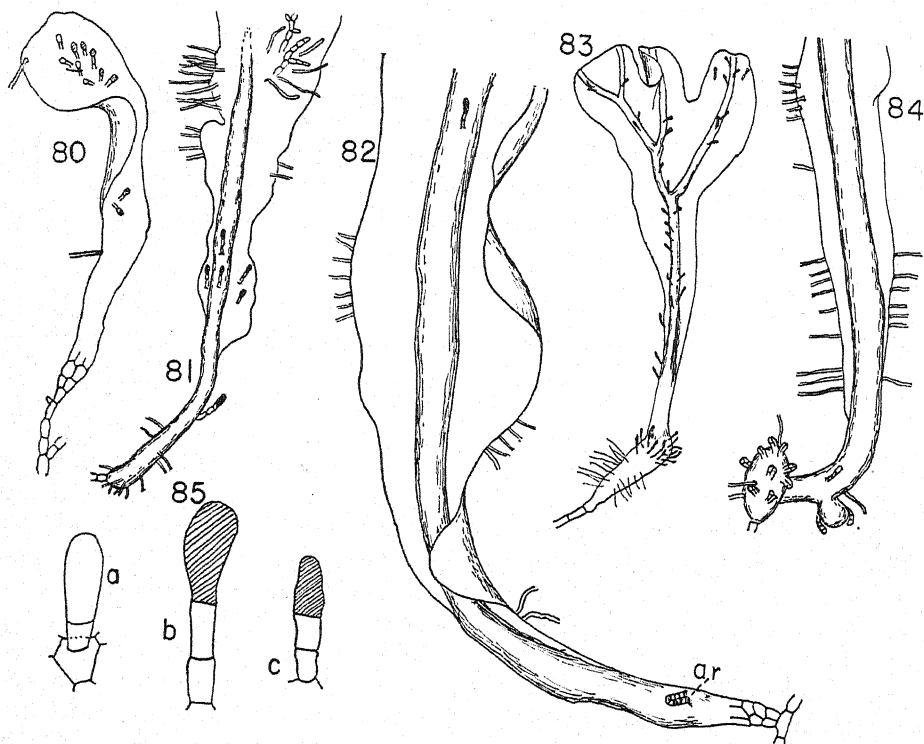
into a flat thallus-like blade or a leaflike blade (figs. 80, 82), while in other cases it arose from an archegoniophore but not from an archegonium (fig. 84). Figure 82 shows an example of an abortive archegonium (*ar*) on the lower part of the thickened portion of the leaflike structure which bore both rhizoids and glandular hairs. Except for this case, all the archegonia appeared normal in structure, and, as active sperms were produced, it seems probable that apogamy in *T. auriculatum* is facultative rather than obligate.

GEMMAE.—When the cultures of *T. auriculatum* were about 3 years old, gemmae appeared on certain branches, especially on the expanded blades which had first appeared in the cultures about 6 months earlier. Gemmae have continued to develop in this species intermittently even though the gametophytes have been producing sex organs and apogamous growths. No gemmae were ever found in *T. bilabiatum*. The gemma begins as a globular green cell on the tip of a specialized cell, the sterigma, which arises as a flask-shaped cell at or near the tip of a filament or plate. The globular cell elongates and undergoes division vertically, obliquely, or horizontally (figs. 86, 87). As the gemma develops, the sterigma tends to lose its chlorophyll and becomes pale, but the gemma remains a vivid green. When gemmae develop in profusion at the tip of a broad expanded branch, they are striking in appearance and suggest a miniature pompom, but they are most easily studied on uniseriate filaments or slender blades. One cell may give rise to two, three, or even more sterigmata or to short filaments which will in turn bear sterigmata and gemmae. GIESENHAGEN (10) presented a drawing of *T. alatum* in which there are at least ten sterigmata on the terminal cell of a

filament. The gemma usually elongates perpendicularly to the long axis of the sterigma. It divides into three, more often four, cells before it is cast off or pushed off (fig. 87). It may be neatly bal-

Discussion

In the revision of the Hymenophyllaceae by COPELAND (6, 7) the four species of *Hymenophyllum* considered here are returned to two PRESL genera—



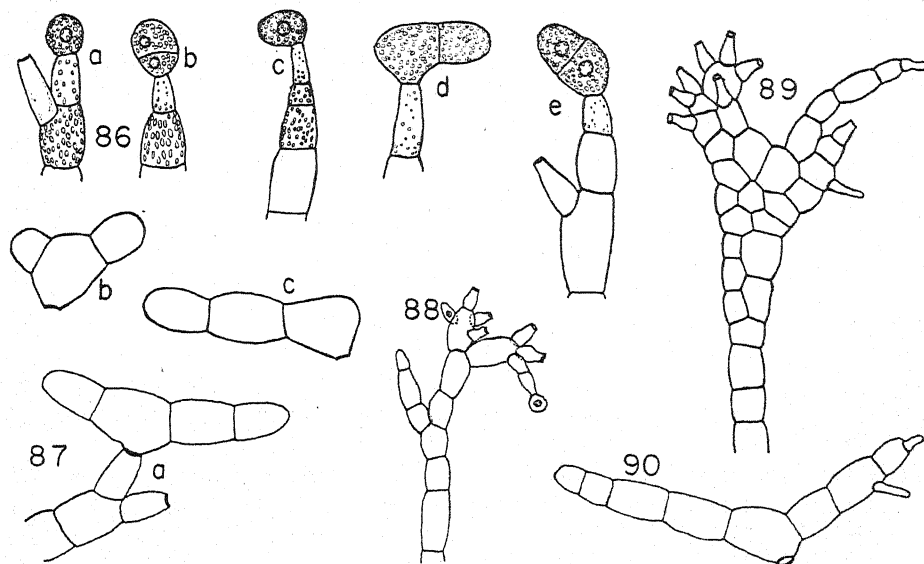
FIGS. 80-85.—*T. auriculatum*, apogamous growths: fig. 80, filament with expanded blade bearing sporophytic hairs; fig. 81, leaflike apogamous growth with filaments, rhizoids, and sporophytic hairs; fig. 82, filament bearing leaflike apogamous growth with abortive archegonium, *ar*, rhizoids, and sporophytic hair; fig. 83, apogamous leaf with sporophytic hairs, rhizoids at base; fig. 84, apogamous leaf which arose from an archegoniophore and produced both rhizoids and sporophytic hairs; fig. 85, glandular sporophytic hairs from apogamous growth in three stages of development.

anced on the sterigma or may develop asymmetrically. Provision is made for its discharge from the sterigma by a change in the character of the terminal wall of the sterigma which separates it from the gemma (fig. 87, *a*). The brown scar in the area of separation remains visible for some time on the detached gemma (figs. 87, *b*, *c*; 90). Rhizoids develop, and the young plant is thus independent.

Mecodium and *Meringium*: *H. blumea-num* Spr. = *Mecodium polyanthos* Sw.; *H. acanthoides* Ros. = *Meringium acanthoides* (v.d.B.) Cope.; *H. holochilum* Chr. = *Meringium holochilum* (v.d.B.) Cope.; *H. kurzii* Prantl is not considered by COPELAND to be distinct from *H. holochilum*. The three species of *Trichomanes* are placed in the two genera *Vandenboschia* Copeland and *Crepidomanes*

Presl: *T. maximum* Bl. = *V. maxima* (Bl.) Cope.; *T. auriculatum* Bl. = *V. auriculata* (Bl.) Cope.; *T. bilabiatum* Nees and Bl. = *Crepidomanes bilabiatum* (Nees and Bl.) Cope. The separation of the *Hymenophyllum* group (the old genus *Hymenophyllum*) into different genera need not be considered in a dis-

(the old genus *Trichomanes*) is sufficiently set apart from that of *Hymenophyllum* in both vegetative and reproductive structures to make it necessary to consider it separately. The species assigned to *Vandenboschia* and *Crepidomanes* do not, however, suggest any generic differences in the gametophyte.



FIGS. 86-90.—*T. auriculatum*, gemmae: fig. 86, stages in development of gemma; fig. 87, older gemmae: a, gemma ready to be cast off; b, c, detached gemmae; figs. 88, 89, portions of gametophyte with young sterigmata and old sterigmata from which gemmae have been cast off; fig. 90, young filament developed from gemma.

cussion of the reproductive structures of the gametophytes, as the differences between these structures are not sufficient in the plants studied and the information about other species is far too meager at the present time. In the ferns we usually find that it is only between subfamilies or groups rather than between genera that differences in the gametophytes are sufficient for special characterization. The group differences and the occasional generic differences are more likely to be seen in the vegetative structures than in the reproductive. The *Trichomanes* group

In an earlier paper dealing with the vegetative structure of these and several other species of the *Hymenophyllaceae* (three in the *Hymenophyllum* and one in the *Trichomanes* group) reasons were given, based chiefly on mode of germination, for looking upon *Hymenophyllum* as the more primitive and *Trichomanes* as derived, in agreement with BOWER (4) and HOLLOWAY (14, 15). HOLLOWAY (15) concluded from his investigation of *Crepidomanes reniforme* (Forst.) Presl (*T. reniforme* Forst.) that this species, which COPELAND stated is perhaps the most

isolated in the family, is more closely related to *Hymenophyllum* than to *Trichomanes*.

The gametophytes of the Hymenophyllaceae, so far as our knowledge extends, are more sharply set apart by their vegetative habit from those of other leptosporangiate ferns than in the structure of their reproductive organs. Such peculiarities as exist in the distribution of sex organs are determined chiefly by the peculiar vegetative habits. The distribution of antheridia on the ribbon-like thallus as well as on the filaments of the *Hymenophyllum* group, and on the filamentous, or less frequently on the plate-like, gametophytes of the *Trichomanes* group, is not unlike that which may be found on ameristic prothalli of other leptosporangiate ferns. The distribution of archegonia presents greater differences. The usual axial archegonial cushion is here replaced by discontinuous marginal cushions in the *Hymenophyllum* group and by discontinuous archegoniophores in the *Trichomanes* group.

The structure of the antheridium of *Hymenophyllum* has been likened to that of the Osmundaceae or of the Gleicheniaceae. This simply means that they are of the primitive type, relatively large and complex with a large sperm output. The sperm output of *Cardiomanes* as indicated by HOLLOWAY's figures and descriptions is even larger than that of any species of the *Hymenophyllum* group which has been described. The antheridia of the *Trichomanes* group are in general smaller and simpler. This may indicate a derived group, but it is also what might reasonably be expected from a smaller gametophyte of simpler organization.

The archegonium conforms in general to the type found in the leptosporangiate ferns in both development and structure.

The long straight neck of the *Hymenophyllum* group is usually associated with primitive ferns, and the shorter neck of the *Trichomanes* group is usually considered a derived type. The straight neck is suggestive of the Osmundaceae. All accounts of the archegonium of *Trichomanes* describe or figure it with a short neck of four cells or less in a row, except BOWER's account of *T. pyxidiferum*, which states that it has regularly five and often six or seven cells in a row. COPELAND has placed *T. pyxidiferum* in his new genus *Vandenboschia* as *V. pyxidifera* (L.) Cope.; he has also transferred *T. auriculatum* to this genus. The length of neck in *Cardiomanes* places it closer to *Hymenophyllum* than to the *Trichomanes* group; HOLLOWAY's figures indicate six to eight cells in a row. This is the usual condition in *Hymenophyllum* and the exception in *Trichomanes*. The regularity of behavior of the cells comprising the axial row is apparently greater than that of the Osmundaceae, Gleicheniaceae, Schizaeaceae, Cyatheaceae, or Dicksoniaceae, according to the results of the present investigation. It should be noted that the ventral canal cell in both *Hymenophyllum* and *Trichomanes* occupies less space in the venter and more in the neck than is usual in the ferns. Although an unusually early division of the basal cell is common in these species, there is a retarded development of the jacket layer of the venter, such as occurs in the Marattiaceae. HOLLOWAY's figures of the mature archegonium of *Cardiomanes* indicate that it also has a meager development of the jacket layer. This peculiarity may be related to the specialized habit—the thin archegonial cushions of *Hymenophyllum* and the tuber-like archegoniophore of *Trichomanes*.

The provision for vegetative propaga-

tion by means of gemmae has apparently been developed more frequently in the *Trichomanes* group than in that of *Hymenophyllum*. Cultural experiments are needed to determine what the conditions are which lead to the formation of gemmae.

The discovery of apogamy in *T. auriculatum* adds a third species to those previously reported for the Hymenophyllaceae: *T. alatum* Sw. which is left in the genus *Trichomanes* in COPELAND's revision, and *T. kaulfussii* Hk. and Grev. which is given as *Macroglena setacea* (v.d.B.) Cope. No case of apogamy has been reported for *Hymenophyllum*.

Summary

1. An account is given of reproduction, both sexual and vegetative, in four species of *Hymenophyllum* which were in culture from 6 to 10 years and of three species of *Trichomanes* in culture from 15 months to 10 years.

2. The gametophytes of *H. holochilum* and *H. kurzii* are monoecious; they bore antheridia when about 20 months old and archegonia a month later. Those of *H. acanthoides* are apparently dioecious; they bore antheridia and archegonia when $3\frac{1}{2}$ years old. Those of *H. blumea-num* are apparently dioecious; they bore archegonia when $3\frac{1}{2}$ years old, but no antheridia were ever found in the cultures.

3. The antheridia of the three species of *Hymenophyllum* are relatively large, complex, and unsymmetrical. They are borne on cells at or near the margin, usually on the lower surface, of the ribbon-like thallus.

4. The archegonia of *Hymenophyllum* develop on meristematic, marginal, rarely central, areas which are two cells thick

and lie close behind the terminal meristem. Permanent tissue one cell thick develops between the successive fertile cushions. The archegonia are usually borne on the ventral surface. A few sporplings appeared in cultures of *H. kurzii*, but fertilization was not observed, and no embryos were found in the preparations.

5. The development of the archegonium follows in general the pattern of the other leptosporangiate ferns. The axial row consists of egg, ventral canal cell, and neck canal cell with two nuclei. The basal cell usually divides early, but the jacket layer of the venter is not well developed when the archegonium matures. The neck is approximately straight with six to nine cells in each row.

6. Regenerated branches appear abundantly on marginal cells of old portions of the thallus in all four species of *Hymenophyllum*.

7. The gametophytes of *T. auriculatum* and *T. bilabiatum* bore antheridia when 9 months old and archegonia about a month later. *T. maximum* bore antheridia at 1 year but died 3 months afterward without producing archegonia.

8. The antheridia of the three species of *Trichomanes* are smaller than those of *Hymenophyllum* and less complex.

9. In *Trichomanes* the archegonia appear on archegoniophores which develop, with or without a stalk, as a lateral, rarely terminal, outgrowth from the filamentous gametophyte. The archegonium develops from a wedge-shaped initial in which the first divisions form the primary neck cell, the central cell, and a pointed basal cell. The basal cell often divides early by a wall perpendicular to the surface of the egg. The neck is straight, and in the two species investigated there are regularly four cells in

each row. Fertilization was not observed, and no normal embryos were found in the cultures.

10. Apogamy was of frequent occurrence in *T. auriculatum* from the time the cultures were 4 years old. The apogamous growths arose from filaments, from bladelike expansions, or from arche-goniophores. They appeared as leaflike blades, often with a midrib consisting in

part of sclerenchymatous tissue. The bladelike structure bore both rhizoids and sporophytic glandular hairs.

11. The gametophytes of *T. auriculatum* produced gemmae on specialized sterigmata either on filamentous branches or, more frequently, at the tip of the bladelike expansions.

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DEVELOPMENT, CELL SHAPE, SUBERIZATION OF INTERNAL SURFACE, AND ABSCISSION IN THE LEAF OF THE VALENCIA ORANGE, CITRUS SINENSIS

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Introduction

An understanding of citrus leaf structure is part of the background necessary for the attack on such current problems as sulfur injury, spray-oil penetration, and the physiology of growth of the tree as a whole. Previous publications listed in the recent monograph of WEBBER and BATCHELOR (50) include numerous studies on specialized leaf structures and functions but no single connected account of the anatomy of the developing leaf. In earlier papers oil glands and crystal idioblasts were discussed in more or less detail (25, 28, 36, 39, 43), while more recent investigations have been concerned with structure in relation to specific function (6, 7, 8, 9, 11, 32, 33, 47, 48, 49). This trend toward physiological anatomy is evident in the investigations on other leaves and has also been discussed in various reviews (1, 2, 3, 4, 5, 19, 29, 46, 51, 52, 53). The present paper, a study of foliar organization, to use WYLIE's term (53), gives a connected account of leaf development in the Valencia orange from primordium to abscission. In it are presented certain findings hitherto unrecorded in the extensive literature on cell shape (13, 14, 15, 22), position of cell walls (37, 38), internal surface (45, 46), and abscission (12, 16, 17, 18, 20, 26, 27, 41).

Material and methods

The leaf of the Valencia orange is unifoliate in type, pinnate-reticulate in

venation (fig. 14), and generally remains on the tree for 2 years or more (50). Normally under orchard conditions it abscises first at the junction of lamina and petiole and later at the node.

Growth-rate studies of length and width of Valencia orange leaves were made on five tagged intact leaves on a vigorous 12-year-old tree growing in the experimental orchard of the Citrus Experiment Station at Riverside. Material for leaf-thickness studies was selected weekly from a population of tagged leaves on the same tree. At the time of tagging all leaves were approximately 5 mm. in length.

Fresh material was used in the anatomical study of all except the youngest stages of growth. This is particularly important in dealing with abscission. Stem tips with leaf primordia were fixed in formalin-acetic-alcohol and thereafter imbedded, sectioned, and stained with Delafield's haematoxylin, safranin, and fast green or with erythrosin and crystal violet (10, 21). Similar material, cleared in hot or boiling chloral hydrate until transparent and mounted in glycerin directly or after treatment with phloroglucin and HCl, demonstrated the origin of provascular and vascular tissue, and also the distribution of oil glands, stomata, calcium oxalate, and hesperidin.

Cell shape and intercellular spaces were studied on paradermal and transverse sections of fresh material cut by

hand or on the freezing microtome, and the results were confirmed by the study of similar sections of imbedded leaves of all ages.

Plasmodesmatal connections were readily demonstrated, though not permanently preserved, by killing in IKI and thereafter irrigating slowly with 80% H_2SO_4 . Continuous observation during this test is essential. Protoplasm, including nuclei and plastids, is stained deep brown. On the addition of the acid, cellulose walls swell, become blue, and sooner or later disintegrate, leaving a perfectly defined protoplasmic reticulum. If the acid enters rapidly, then the plasmodesmata become very much stretched, but if it is allowed to percolate slowly under the cover slip, then the protoplasmic strands remain approximately their normal length, the thickness of the cell wall.

The IKI- H_2SO_4 method was also used in demonstrating the occurrence of suberin lamellae in intercellular spaces in the middle lamellae, and as a tertiary layer within the cell.

Standard microchemical tests were used in studying the distribution of starch, calcium oxalate, and fatty substances (44). Steimetz fluid was useful occasionally in the examination of fresh material (21). Preliminary immersion overnight in IKI accentuated clearly and simultaneously the distribution of starch, fat, lignin, and suberin.

Observations

The development of a leaf is a continuous process, but, for the sake of clarity in description, it will be discussed under the following headings: (a) resting bud and meristematic primordia; (b) expanding leaf; (c) maturing leaf; and (d) leaf fall. The first three phases—meristematic, expanding, and maturing—are direct-

ly related to the growth regions of the shoot—apical meristem, region of elongation and differentiation of primary tissues, and region of secondary thickening—while the fourth phase is followed by leaf-scar formation.

RESTING BUD AND MERISTEMATIC PRIMORDIA

Between "flushes of growth" the resting bud is covered by three to five small green bud scales which are roughly triangular in outline, swollen at the base, and blunt or tapering at the apex. Trichomes one- or many-celled, may occur along the scale margins (fig. 15). The scale leaves may occasionally dry out and appear shriveled and brownish in color. Within the bud scales lies the apical dome, encircled by eight leaf primordia. The number of leaf primordia is predictable from the phyllotaxy, $\frac{3}{8}$, as evident in older twigs.

At the beginning of growth as the scales are shed, the leaf primordia range in size from the microscopic arc of leaf 1 to the slender blade and petiole, approximately 2 mm. in length, of leaf 8 (figs. 2, 3, 12). In primordium 2, which overlaps the apical dome (fig. 4), the median provascular strand differentiates acropetally and is flanked by vacuolating dividing cells. Spiral elements appear at the base of leaf 3, trichomes at its apex (figs. 5, 6). The initiation of blade development appears in slight lateral swellings of primordium 4, and in this primordium is also foreshadowed the differentiation into lamina and petiole (figs. 7, 13). The midrib extends as far as the first clearly defined terminal oil gland. Stomata are present on the dorsal surface. In primordium 5 the midrib forks right and left of

* The terms "flushes of growth," "growth flushes," or "growth cycles" indicate the times of year, spring or fall, when new leaves are produced.

the terminal oil gland. The asymmetry characteristic in later growth is evident in the unilateral development of spiral elements (figs. 8, 11).

Up to this point the leaf primordia consist mainly of midrib tissue, but in primordium 6 the flanges of marginal meristem become active, and the blade, at this stage folded, begins to appear (fig. 9). The future absciss layer between blade and stalk is indicated by a constriction. Trichomes are more or less

EXPANDING LEAF

Although the leaves of deciduous plants such as *Vitis* expand and mature in the relatively short period of 40 days (23), the leaves of evergreen trees such as *Citrus* and *Abies* expand and mature much more slowly. In *Abies lasiocarpa*, for instance, growth in blade length continues for more than 2 years (40). In order to determine the period of growth of Valencia orange leaves, five leaves approximately 5 mm. in length (this size of

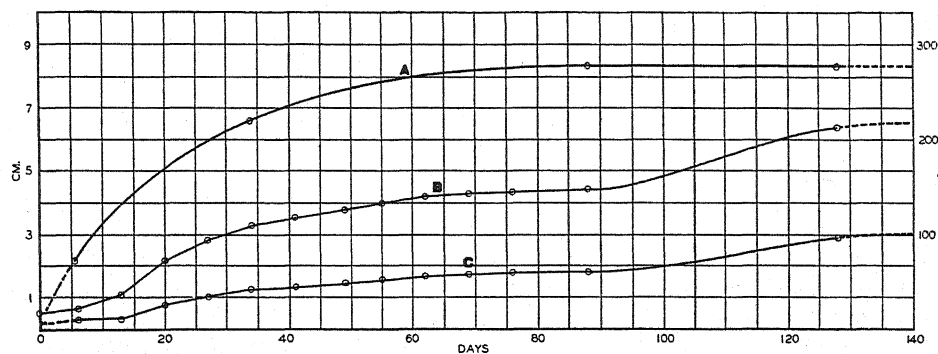


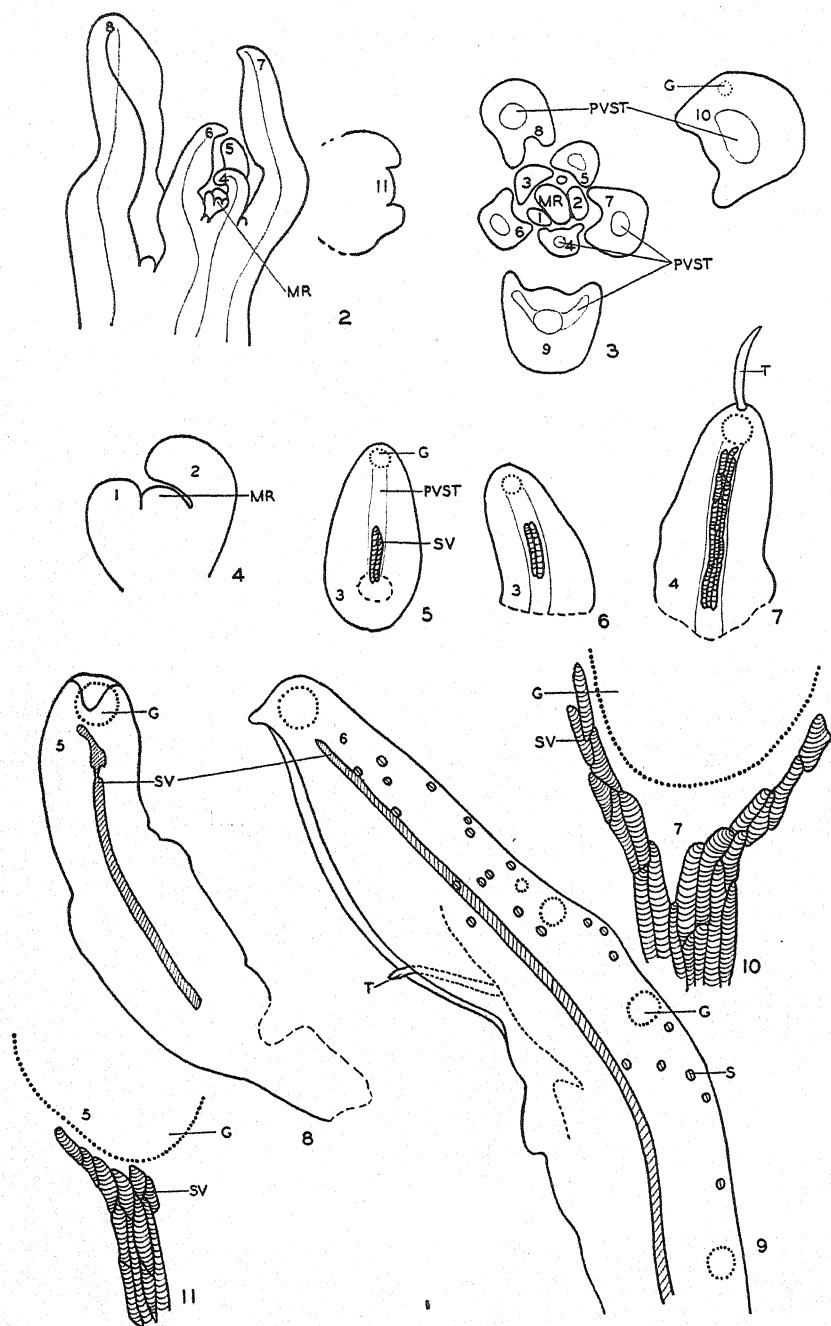
FIG. 1.—Average corresponding increases in dimensions of five Valencia orange leaves 0.6 cm. in length from spring until fall flush of growth. A, leaf thickness. B, blade length. C, blade width. Blade length and width are given on left ordinate in centimeters; blade thickness on right ordinate in microns; time in days on abscissa.

frequent on the ventral surface and are most conspicuous at the proximal end of the lamina and on the distal end of the petiole. Additional oil glands are apparent—also stomata in various stages of differentiation on the dorsal surface. Spiral vessels are now differentiated in both prongs of the apical fork of the midrib surrounding the terminal oil gland (fig. 10).

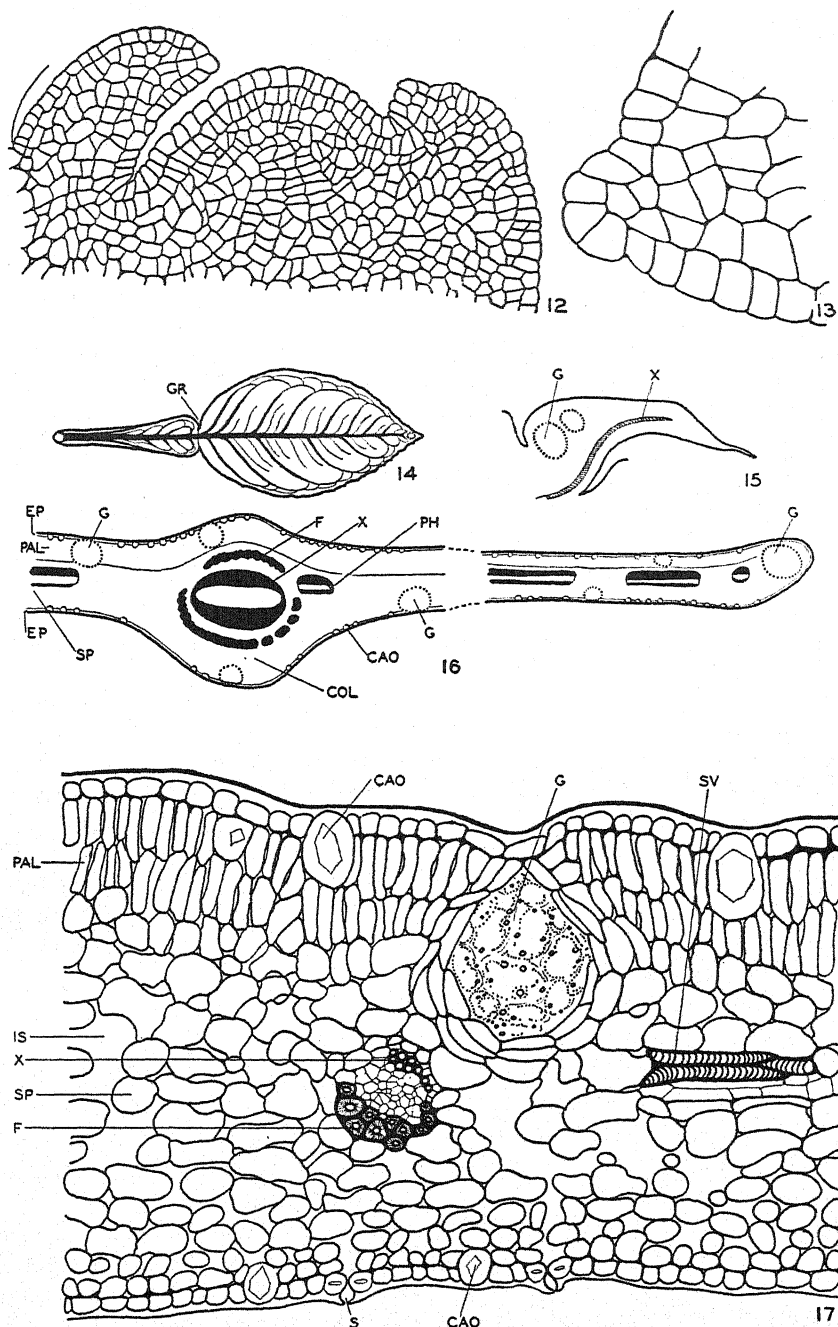
Primordia 7 and 8, the remaining leaves of the meristematic phase of growth, measure 1.8 and 1.9 mm. long, respectively, and in them further development of blade, vascular tissue, oil glands, and stomata is apparent.

leaf is the smallest which can be handled without injury) were tagged in spring, and their length and width were measured and recorded weekly until early fall. The following measurements of growth therefore date from the time that the leaf had attained a length of 5 mm. At the time of each weekly measurement five other leaves of approximately the same size were picked, fixed, imbedded, and sectioned in order to determine blade thickness. All measurements of blade thickness were made near the center of the lamina.

Growth in blade length and width initiated during the spring flush of growth



FIGS. 2-11.—Terminal bud. *G*, gland; *MR*, meristem; *PVST*, provascular strand; *S*, stoma; *SV*, spiral vessel; *T*, trichome. Fig. 2, cleared stem apex showing position of leaf primordia. Fig. 3, transection of similar stem apex (camera lucida). Figs. 4-9, primordia 1-6 showing beginning of vascular differentiation. Figs. 10, 11, tip of midrib of primordia.



FIGS. 12-17.—Fig. 12, detail of longitudinal section of apical meristem, showing primordium 1 and base of 2 (camera lucida). Fig. 13, detail of beginning of lamina of primordium 4 (camera lucida). Figs. 14-17, general structure of leaf. CAO, calcium oxalate; COL, collenchyma; EP, epidermis; F, fiber; G, gland; GR, groove; IS, intercellular space; PAL, palisade; PH, phloem; S, stoma; SP, spongy mesophyll; SV, spiral vessel; X, xylem. Fig. 14, diagram of mature leaf, showing principal veins. Fig. 15, diagram of leaf scale. Fig. 16, diagram of transection of mature leaf, showing distribution of tissues. Fig. 17, transection of mature leaf, showing small vein and other tissues.

continued for about 130 days (fig. 1). Two marked periods of accelerated growth occurred within this time, the first after 13 days. This was followed by a period of decreased rate of expansion, which continued until about the ninetieth day. A second period of rapid expansion then began, which continued until the leaf was approximately 128 days older than the initial 5-mm. stage. The two periods of rapid expansion coincide with the spring and fall growth flushes. The rate of increase in blade length is consistently a little greater than the rate of increase in blade width.

TABLE 1

MEASUREMENTS IN MICRONS OF TYPICAL INDIVIDUAL CELLS IN YOUNGER AND OLDER LEAVES AS SEEN IN TRANSECTION OF MID-LEAF

	Epidermis	Mesophyll
Young leaf. (0.7×0.2 cm.)	14×14	Undifferentiated 15×15
Old leaf. (11×5.8 cm.)	19×15	Palisade 28×10 Spongy 38×38

The rate of increase in leaf thickness during the first 65 days is relatively more rapid than is the rate of increase in either blade length or width. Full thickness is attained in about 80 days (fig. 1). The tree is thus provided with a potentially highly functional new photosynthetic leaf area for the winter season. A study of the rates of expansion in leaf dimensions and their timing indicates that fundamental changes occur in the sizes of cells of the various laminar tissues. That interaction of rate and time of cell expansion determines to a large degree cell shape within the leaf will be shown later.

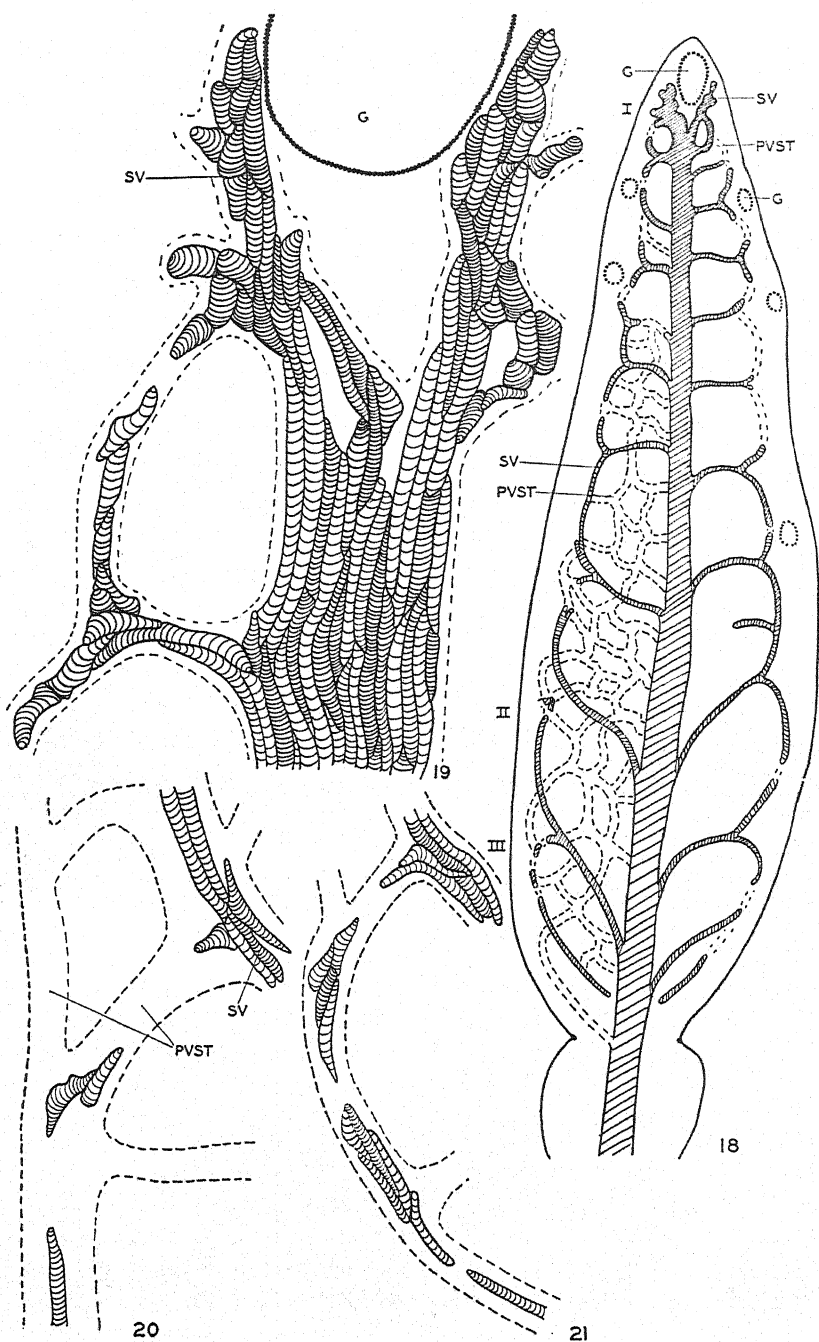
The expanding leaf reaches its full size during the second phase of development.

During this phase it is light-green in color, soft in texture, and wilts immediately upon picking. The lamina and winged petiole are delimited by the groove marking the future laminar abscission layer. The midrib projects on the dorsal surface and is marked by swellings at the junction of leaf blade and stalk and also at the leaf base. Orientation of the leaf in relation to light frequency results in torsion of the leaf base.

During this period of expansion, differentiation of primary tissues is completed. Palisade and spongy mesophyll appear and, concurrently, the intercellular spaces. Stomata, oil glands, calcium oxalate, idioblasts, and vascular tissues continue to develop (figs. 16, 17). Increase in cell size is indicated in table 1.

VASCULAR TISSUE AND ABSCISSION ZONES.—During the second phase of development the primary vascular system is completed (fig. 18). At the leaf tip, elongate spiral elements differentiate, first, along the vascular fork. Irregular, shorter, and thicker cells appear later and are frequently roughly isodiametric in outline and project from the main surface of the vein. Lateral veins arise basipetally and asymmetrically, the first proximal to the apical oil gland (fig. 19). Within the lateral provascular strands, differentiation of spiral elements proceeds in continuous linear series from midrib to leaf margin and thereafter in both directions toward leaf tip and leaf base. At the same time discontinuous foci of spiral vessel differentiation and lignification appear along the marginal veins (figs. 20, 21). Provascular and vascular differentiation keeps pace with oil-gland formation, and developing veinlets may be traced from the marginal veins to the oil-gland bases.

While the main pattern of venation—midrib, laterals, and marginal veins—is



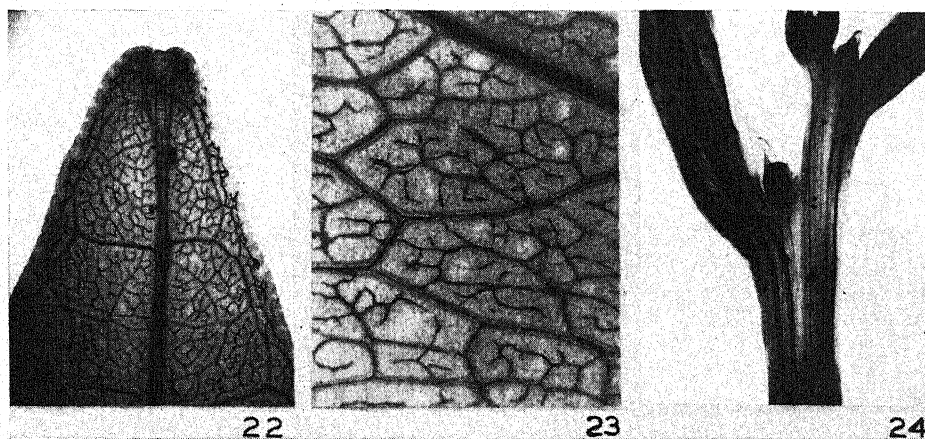
FIGS. 18-21.—Venation. G, gland; PVST, provascular strand; SV, spiral vessel. Fig. 18, cleared leaf 4.6 mm. long, showing midrib cleft at leaf tip, lignification of midrib and major laterals, differentiating provascular network and glands. Fig. 19, detail of leaf tip (level I). Figs. 20, 21, discontinuous lignification near leaf margin (fig. 20, level II; fig. 21, level III).

being defined, the interlateral areas differentiate into a mosaic of vein islets. The size of vein islets or areoles is extremely variable (figs. 22, 23). In the smaller veinlets, spiral elements may differentiate in linear series from midrib, laterals, or marginal veins. As in the case of the marginal veins, however, discontinuous foci of lignification also appear.

The nodal structure is readily seen in cleared material (fig. 24). Three leaf

includes sieve tubes, companion cells, and phloem parenchyma. The xylem consists, at first, of primary spiral vessels and occasional tracheids only, while secondary pitted vessels, tracheids, and xylem fibers differentiate later. As in the stem, the outer pith is made up of smaller elements which grade into the larger cells of the central core.

The anatomy of the midrib is modified in the laminar and nodal abscission re-

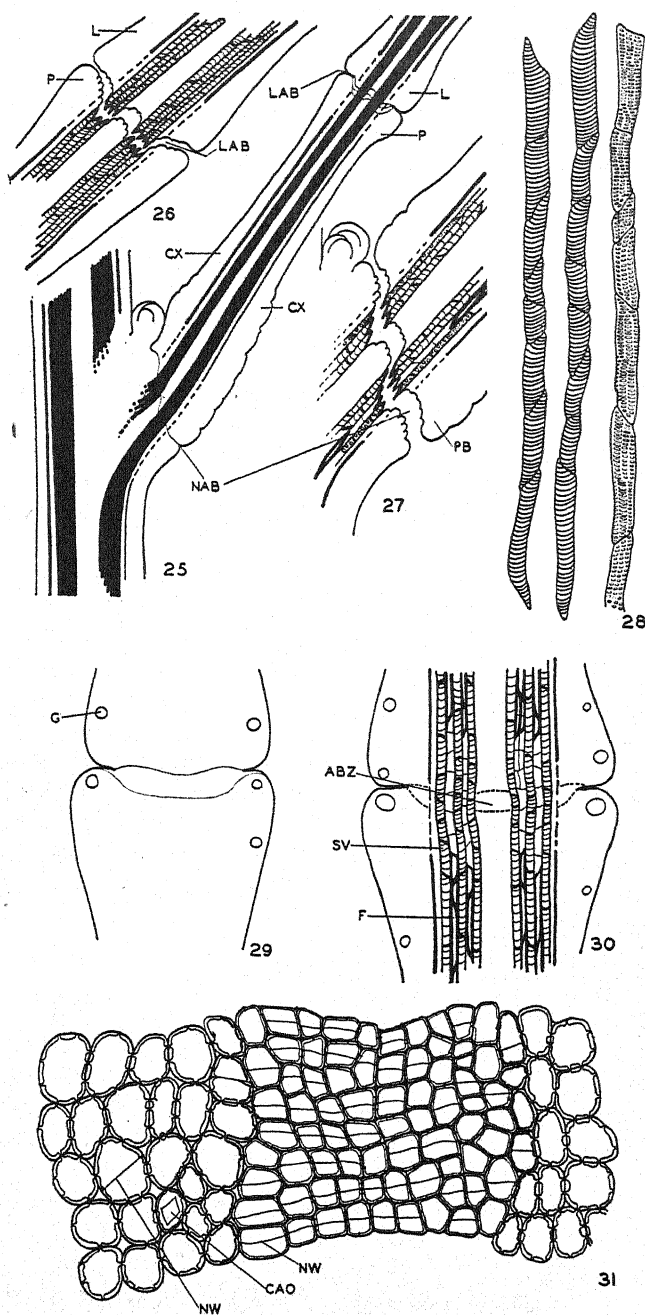


FIGS. 22-24.—Venation and node. Fig. 22, cleared mature leaf tip. Light spots indicate oil glands particularly clear along leaf margin. Fig. 23, detail of leaf areoles from mid-leaf. Fig. 24, cleared node showing leaf traces.

traces enter the leaf base, the largest of which—the median—is flanked by two smaller laterals. Within 1 mm. of the base, the traces unite to form a siphonostele which continues throughout the petiole and the greater part of the midrib. The outline of the stele, except in the laminar abscission region, is roughly semicircular, the diameter ventral in position (fig. 80). The stele is delimited by a starch sheath and by a more or less continuous cylinder of pericyclic fibers which become lignified except in the laminar and nodal abscission regions. The vascular tissues of the midrib are similar to those in the stem. The phloem

gions (figs. 25-27, 29, 30). Structural weakness is evident in both—in the lack of lignification of the pericyclic fibers, the shortness of the vessel segments (fig. 28), and the increase in the vascular parenchyma. In older leaves when secondary thickening begins concurrently in stem and petiole, the inherent weakness is further accentuated in the comparative lack of xylem fibers and their unlignified condition if present.

The laminar abscission region differs from the nodal in the presence of a definite abscission zone (fig. 31). This band of cells, approximately ten layers deep, lies at the distal end of the petiole almost



FIGS. 25-31.—Abscission. *ABZ*, abscission zone; *CX*, cortex; *F*, fiber; *G*, gland; *L*, lamina; *LAB*, laminar abscission zone; *NAB*, nodal abscission plane; *P*, petiole; *PB*, petiole base; *SV*, spiral vessel. Fig. 25, diagram of longisection through lamina, petiole, and leaf base. Figs. 26, 27, diagrams of longisections through abscission zones, laminar and nodal, respectively. Fig. 28, camera lucida drawing of vessels from abscission zones, showing short vessel-segments. Figs. 29, 30, diagrams of paradermal sections below and through midrib in laminar-petiole abscission region. Fig. 31, soft green leaf, cortex of midrib; paradermal section showing dividing cells.

level with the groove and is distinguished by the smaller size of cells, lack of intercellular spaces, and comparatively prolonged meristematic activity. Abscission eventually occurs along the separation layer, the distal face of the absciss zone.

In contrast, no definitive abscission tissue is present in the nodal abscission region. The line of rupture is first indicated in older leaves by differential distribution of starch and intercellular spaces.

EPIDERMIS AND CORTEX.—Epidermal cells are polygonal in outline and more or less uniform in size. The outer walls are cutinized and coated with wax, which is secreted through minute canals similar to those in the fruit rind. On treatment of sections with $\text{IKI-H}_2\text{SO}_4$ it is evident that these canals are lined with protoplasmic threads similar to plasmodesmata. Protoplasmic activity within these channels is presumably responsible for the maintenance of the waxy surface (figs. 40, 41).

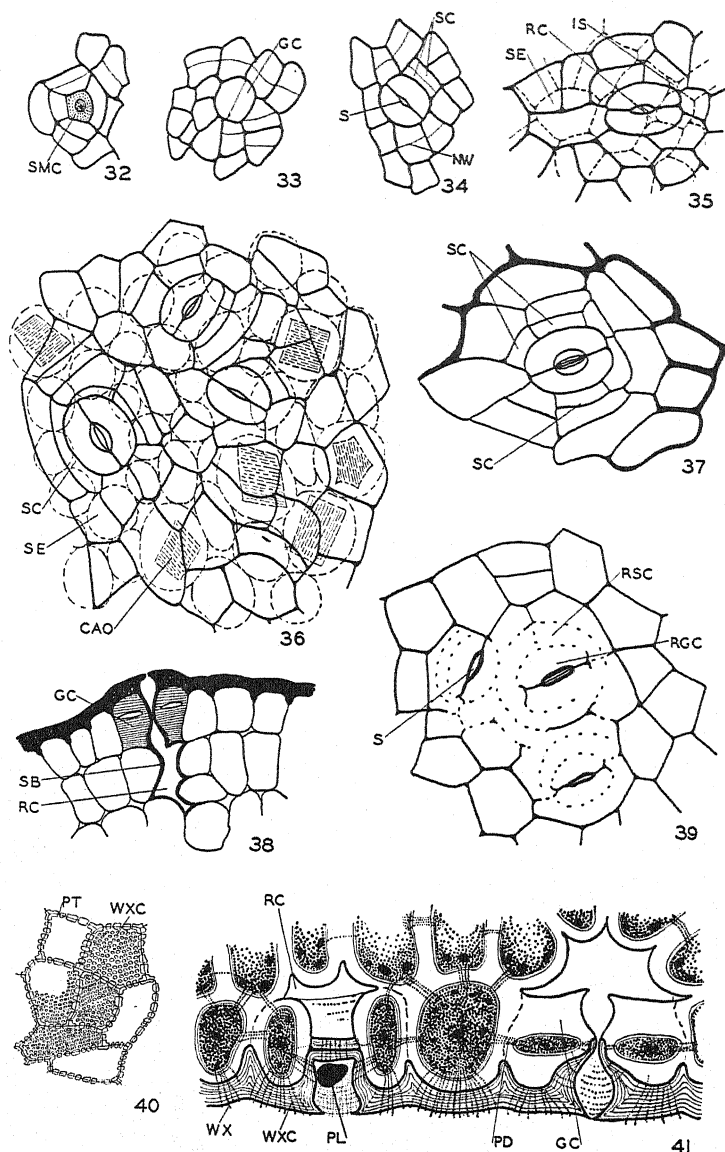
The comparative uniformity of the epidermal mosaic is broken by stomatal areas and gland cover cells. Stomatal development may be followed from the leaf primordia onward and resembles that seen in the fruit rind (figs. 32–38). The stomatal mother cell is first distinguished by its isodiametric outline and the comparative density of the protoplast. When cell division occurs, the daughter cells assume the characteristic outline of guard cells. Thickening and cutinization along the median third of the dividing wall mark the position of the future stoma. Meanwhile, the surrounding cells divide by walls roughly parallel to the circumference of the guard cells. Ultimately, the mature guard cell is bordered more or less completely by a zone of narrow accessory cells similar to, but fewer in number than, those in the fruit. As in

the fruit, the accessory cells are but thinly cutinized and disintegrate very soon upon treatment with $\text{IKI-H}_2\text{SO}_4$, while the rest of the outer wall of the epidermis persists intact (fig. 39). Stomata are numerous on the lower surface of the leaf while only a few appear along the midrib on the upper. Variation in stomatal number and distribution has been discussed in numerous previous papers (8, 32).

Gland cover cells are distinguishable principally by their translucence, resulting from the increased oil content of the protoplast and walls, and also by their roughly radial orientation.

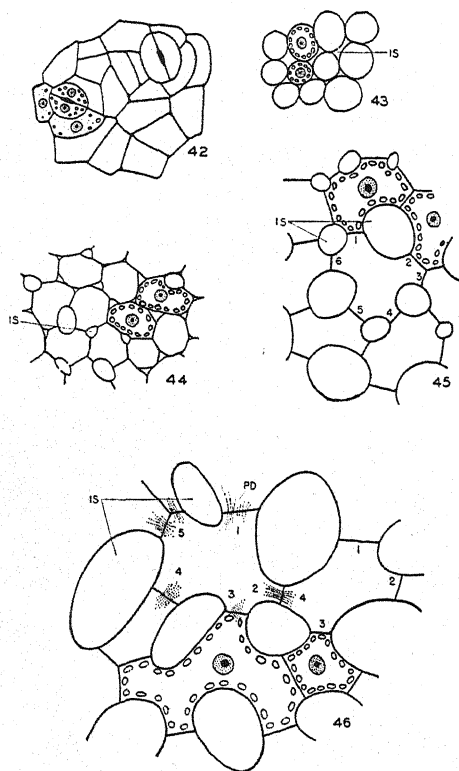
The cortex of the petiole includes chlorenchyma and collenchyma. The former consists of comparatively short palisade cells continuous with the palisade tissue of the petiolar wings. Collenchyma occurs as longitudinal strands above and below the midrib, at the level of the laminar groove, and also at the leaf base, where it encircles the stele.

INTERNAL SURFACE, SPONGY AND PALISADE PARENCHYMA.—Differentiation of spongy and palisade parenchyma and, concurrently, of intercellular spaces, is completed during the second phase of development. In figures 42–46 are indicated the cross-sectional areas of intercellular spaces at various levels of the fully expanded leaf. These areas are of maximum size at vein level, i.e., in mid-mesophyll, near the center of the leaf areoles. Development of intercellular space depends on the differential duration of cell division in the various tissues. Cell division ceases, first, at vein level, and the cells of the middle mesophyll thereafter keep pace with continuing leaf expansion by stretching growth only and differentiate as the large and characteristic armed cells of the spongy mesophyll. Meanwhile, in the remaining tissues—upper epidermis, palisade, lower spongy



FIGS. 32-41.—Differentiation of stomata and epidermal cells. *CAO*, calcium oxalate; *GC*, guard cell; *IS*, intercellular space; *NW*, new wall; *PD*, plasmodesmata; *PL*, plug; *PT*, pit; *RC*, respiratory chamber; *RGC*, remains of guard cell; *S*, stoma; *SB*, suberin; *SC*, subsidiary cell; *SE*, subepidermal cell; *SMC*, stomatal mother cell; *WX*, wax; *WXC*, wax canal. Figs. 32, 33, 34, surface of epidermis; stomatal mother cell and two early stages of differentiation; subsidiary cells in fig. 34; size of mother cell $9 \times 8 \mu$. Figs. 35, 36, further stages of differentiation, with underlying hypodermal cells, intercellular spaces, and calcium oxalate idioblasts. Fig. 37, mature stoma surrounded by subsidiary cells, lightly cutinized in comparison with other cells. Fig. 38, mature leaf, transection of stoma, showing suberization of intercellular space. Fig. 39, mature stomatal area after treatment with $\text{IKI-H}_2\text{SO}_4$, showing dissolution of subsidiary cells. Fig. 40, epidermal cells, surface view, after treatment with H_2SO_4 , showing wax canals. Fig. 41, mature leaf, transection of stomata during treatment with $\text{IKI-H}_2\text{SO}_4$, showing suberization of respiratory chamber, stomatal plug, wax canals, stratification of epidermis, and plasmodesmata.

mesophyll, and lower epidermis—cell division continues considerably longer. As a result, the cells of these tissues, in their small size, contrast markedly with those of the central spongy layers.



FIGS. 42-46.—Tissues of mature leaf at different levels to show intercellular spaces. *IS*, intercellular space; *PD*, plasmodesmata. Fig. 42, lower epidermis. Fig. 43, hypodermis. Fig. 44, lower spongy mesophyll. Figs. 45, 46, mid-mesophyll.

In meristematic tissues new cell walls are distinguished first as tenuous membranes ensheathed in a protoplasmic plate. As they become clearly defined, they are seen invariably to become anchored to the wall of the parent cell in plasmodesmatal areas (figs. III-III2).

The cells of the palisade and spongy tissues, though differing markedly in mature form, nevertheless follow the

same fundamental pattern of development next to be considered in detail.

In spongy tissue two principal cell shapes occur, the eight-armed cell (figs. 47-57) and the six-lobed cell (figs. 58-68). The eight-armed cells occur near the center of the areoles at vein level, while the six-lobed cells compose the marginal tissue closer to the veins and the lower spongy parenchyma.

The meristematic cells of the leaf primordia are typically tetrakaidekahedral in outline (30). Cell division continues while the cells expand and become spherical and while, at the same time, intercellular spaces appear. All cells, however, remain interconnected by plasmodesmata, eight in the case of the typical spherical element. Gradually, cell division slows down and stops, first in mid-areole at vein level. The mid-mesophyll tissue thereafter keeps pace with growth in area of the leaf as a whole, by expansion of both cells and intercellular spaces. The erstwhile spherical cells expand more or less evenly in all directions, becoming, first of all, eight-lobed and, finally, eight-armed. The mid-mesophyll in mid-areole is the zone containing the largest intercellular spaces found within the leaf. The eight-armed cells are similar in form, though not in size, to the typical albedo cells of the citrus rind.

The shape of mature cells is determined by the time of cell division in relation to the expansion of the lamina as a whole. In considering differentiation of the six-lobed cell, it is necessary to study the effects of cell division on the number of cell contacts. In meristematic tissue, when active cell division occurs, the number of contacts is temporarily altered to more or less than eight. Thus in optical section any one meristematic cell may connect with three, four, five, seven, or more adjacent elements. During this pe-

riod of active division the fundamental hexagonal pattern of structure remains obvious in the distribution of the larger and older and of the smaller and younger intercellular spaces and also in the constantly recurring restoration, during growth, of the initial six-cell contacts. The relative time of division, more or less recent, is indicated by the thinness and orientation of the new cell walls. If a typical spherical cell with eight contacts divides twice in rapid succession and if the adjacent cells are not at this moment dividing, then three daughter cells result, each of which is in contact with six others. Sooner or later thereafter the original intercellular spaces enlarge, and, in the center of the daughter cells, a new space appears. Three daughter cells, six-lobed, in contact with four cells (optical section) and with two others above and below, are illustrated in the photographs of the plastic models constructed by one of us from camera lucida drawings of the cells of a lemon leaf vein islet (figs. 69, 70).

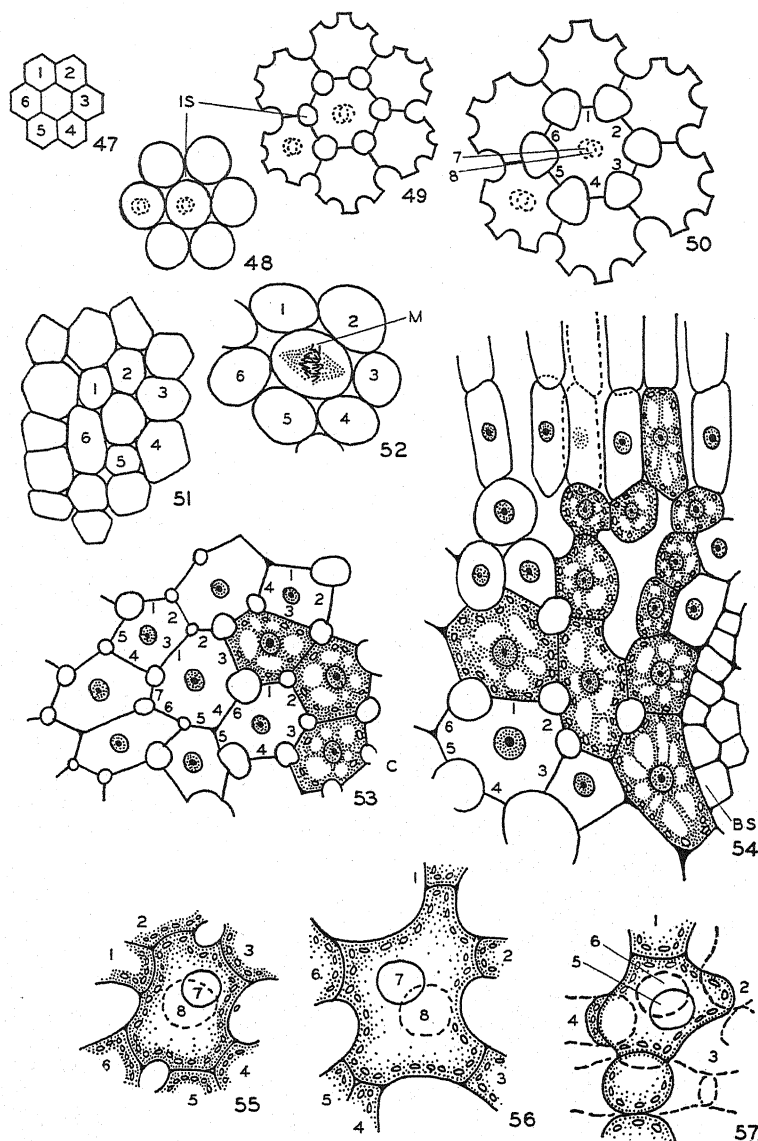
In the mature palisade (figs. 71-77) similar typical cell shapes are distinguished, such as eight-contact and six-contact cells. These are the counterparts of the eight-armed and six-lobed cells of the spongy mesophyll, but in them the plasmodesmata pass through barely defined papillae or low ridges instead of through well-developed arms or lobes. In palisade tissue, cell division continues later than in the spongy tissue, with the result that all cells are considerably smaller.

The pattern of development in palisade tissue resembles that in spongy parenchyma. Palisade cells, like spongy, differentiate from tetrakaidekahedra which are indistinguishable, except by position, from future spongy mesophyll elements in the leaf primordia. The pali-

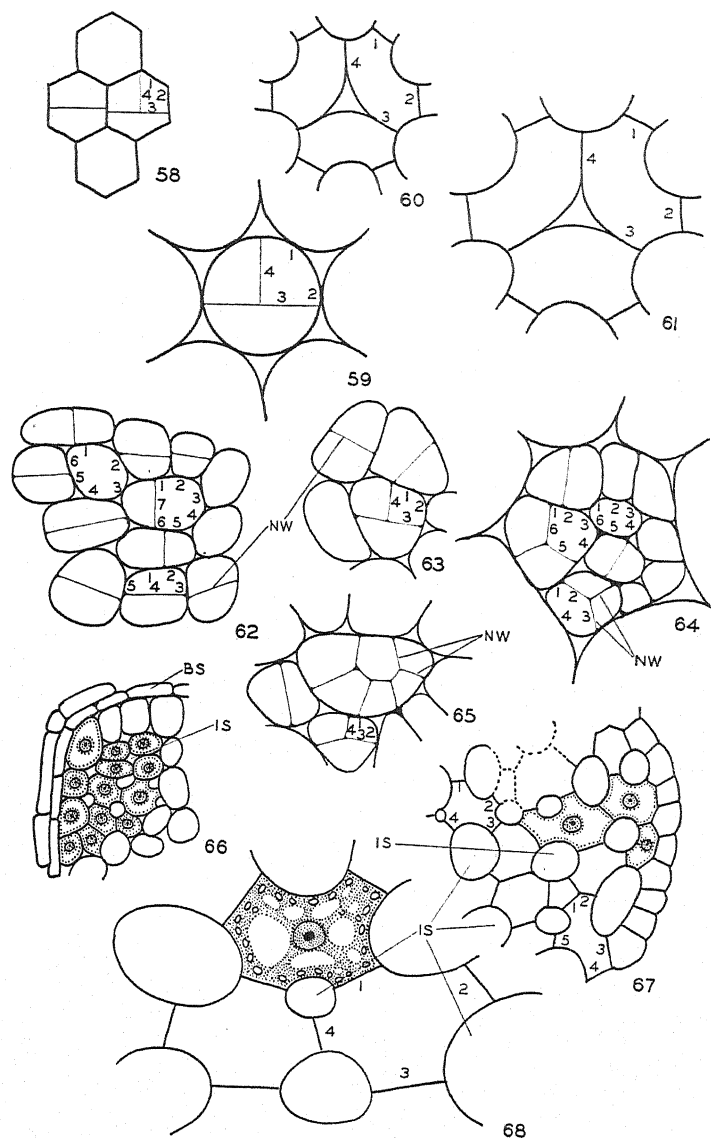
sade polyhedra become spherical, and intercellular spaces develop, while plasmodesmata, typically eight per sphere, maintain intercellular connections. Very soon after the appearance of intercellular spaces, divergence in development occurs. When the leaf measures ± 3 cm. in length, the palisade cells elongate, become ellipsoidal, and assume their characteristic outline.

In the mature leaf the palisade cells vary greatly in diameter, and the diameter of the largest may measure more than twice that of the smallest, $\pm 15 \mu$ in contrast to $\pm 6 \mu$ (figs. 73, 74). The larger cells, in general, occur near mid-areole, while the smaller line the areolar margins. The time of cell division in relation to leaf expansion as a whole is responsible for the number of cell contacts. If a palisade cell differentiates early in the middle of a leaf areole and is relatively free to expand in width as in length, then such a cell remains connected by plasmodesmata with eight other cells. In optical sections parallel to the leaf surface these typical eight-contact cells appear surrounded by six others. If, on the other hand, cell division occurs relatively late and free expansion is not possible, then the number of contacts is altered to more or less than eight.

Plasmodesmata in the mature palisade cells are of two types, pit and diffuse (figs. 75, 76, 77). The former occur commonly near the outer and lower ends of the cell, terminal or subterminal in position. The latter, the diffuse, consist of very numerous slender single strands which may interlock adjacent cells throughout the entire vertical length of the cell wall. Presumably they either may result from separation of initial strands during cell-wall extension or may originate directly during the latest division of the cylindrical cells.



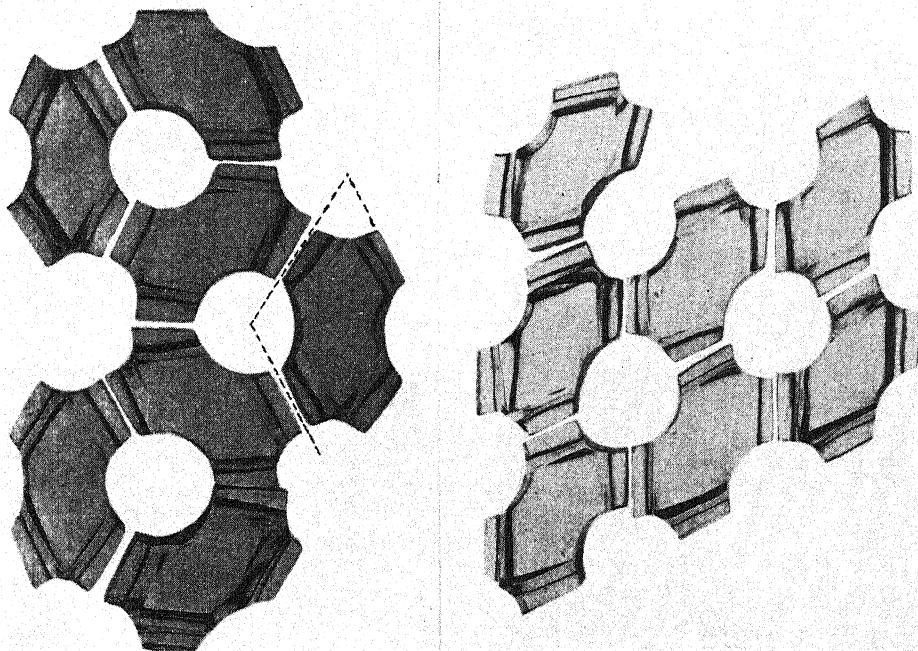
FIGS. 47-57.—Differentiation of eight-armed cells. *IS*, intercellular space; *M*, mitosis. Figs. 47, 48, diagrams of transection of tetrakaidekahedra and spheres. Figs. 49, 50, diagrams of increased intercellular spaces and resultant lobe and branch formation. Figs. 51-57, camera lucida drawings for comparison with diagrams. Figs. 51, 52, transections of soft green leaf, meristematic stage. Cf. figs. 47, 48. Fig. 53, tangential section of lower mesophyll of mature leaf, showing lobed cells (cf. fig. 49). Fig. 54, transection of mature leaf showing armed cells of spongy mesophyll (cf. fig. 50). Figs. 55, 56, eight-armed cells from mid-mesophyll. Fig. 57, six-armed cell.



FIGS. 58-68.—Variation in cell contact number, due to cell division and differentiation of six-lobed cells. *BS*, bundle sheath; *IS*, intercellular space; *NW*, new wall. Figs. 58-61, diagrams. Fig. 58, transection of tetrakaidekahedra in which division is taking place by two successive walls. Fig. 59, transection of sphere formed as tetrakaidekahedra round off. Figs. 60, 61, further development of intercellular space. Figs. 62-65, camera lucida drawings for comparison with figs. 58-61. Figs. 62-65, transections of soft green leaves in meristematic stage, showing formation of new cell walls. Figs. 66, 67, paradermal sections of two stages in differentiation of mesophyll. Fig. 68, paradermal section of mesophyll (cf. figs. 59, 60, 61).

ERGASTIC SUBSTANCES—The principal ergastic substances which may be distinguished microscopically are starch, calcium oxalate, fatty substances, and the usual wall materials, including suber-

mottled, the areoles becoming lighter and the veins a darker green (figs. 78, 79). At this time of year (November), mottling results from differences in chloroplast size and starch content. In light areas the



FIGS. 69, 70.—Photographs of lucite models of spongy mesophyll cells of lemon leaf; cells made to scale from camera lucida drawings. Fig. 69 (*left*), triad arrangement around vertically inclined intercellular spaces. Four lateral lobes and one of two vertical lobes of each cell are shown. Lateral lobe faces are at 120° and 60° angles. Fig. 70 (*right*), cells arranged in fours around intercellular spaces. Replacement of single cell oriented along intercellular space in longer dimension in this grouping by two cells oriented on intercellular space in shorter dimension results in group of five cells bordering intercellular space. When all cells bordering intercellular space are oriented with their shorter dimensions on intercellular space, six cells constitute unit grouping.

in and wax. Hesperidin crystallizes out upon partial dehydration of cells in alcohol or other reagents but is not considered in this paper.

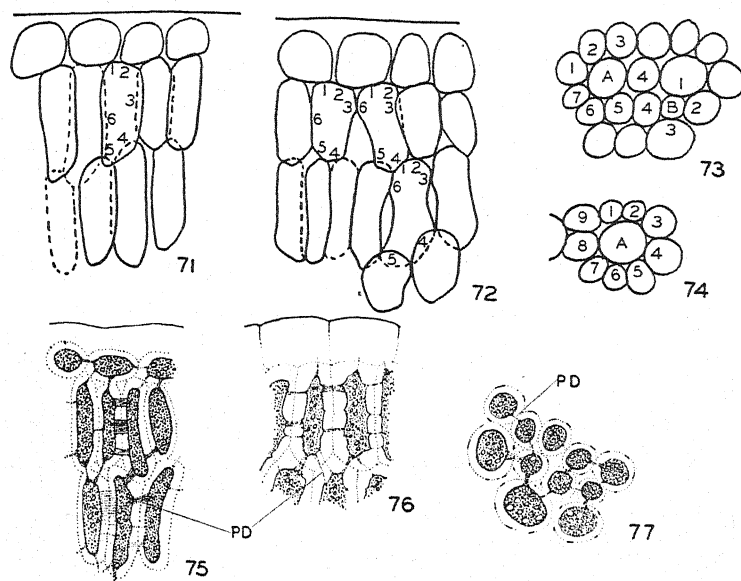
During the second phase of growth, starch appears as usual in the chlorenchyma of lamina and petiole, in the guard cells, and in the bundle sheaths. Toward the end of the period the leaf becomes

plastids in mid-areole are comparatively small, completely filled with starch—one single grain only, as a rule—and resemble those in young cells in that the majority are still clustered around the nucleus. In contrast, chloroplasts in the darker areas are almost twice the size of the former, are generally parietal in position, and contain only the usual minute

transitory starch granules. As the leaf becomes uniformly dark-green, reserve starch accumulates throughout the leaf and is particularly conspicuous in bundle sheath, rays, and pith of the midribs and larger veins (figs. 80, 81, 84-87).

Mature crystal idioblasts of citrus (figs. 90-96) have been described repeat-

limit of visibility. Sheath and crystal are suspended near the center of the cell by protoplasmic strands anchored to the wall by plasmodesmata. Sooner or later, crystals increase in size and more or less completely fill the cell cavity. The characteristic cellulose sheath keeps pace with crystal growth and becomes heavy



FIGS. 71-77. Cell shape in palisade tissue. PD, plasmodesmata. Figs. 71, 72, mature leaf, transection, indicating cell contacts (camera lucida). Figs. 73, 74, similar leaf, paradermal section, showing variation of diameter of palisade cells and variation in number of cell contacts around typical cells A, B (camera lucida). Figs. 75-77, transections and paradermal section through palisade tissue during IKI- H_2SO_4 test, showing plasmodesmatal connections (camera lucida).

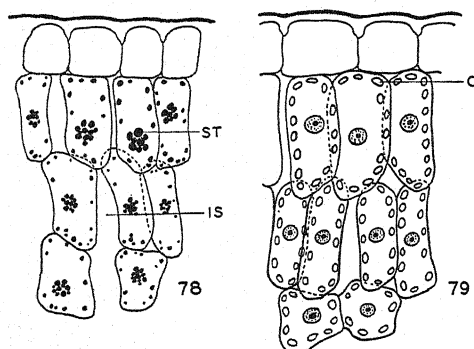
edly in earlier papers. It will suffice at this time to outline their development and indicate their distribution. In young leaves ± 2 cm. in length, idioblasts are recognized in upper and lower hypodermis by their larger size and denser protoplasmic content. Minute crystals are soon apparent within one or the other of the protoplasmic strands, generally near the center of the cell. By the time that the crystals measure from 4 to 5 μ , they are tabular in outline and are incased within a tenuous sheath which is at the

at the crystal base but remains tenuous at the apex. Meanwhile, the wall of the idioblast may thicken until sheath and wall are in contact at one or more points. Plasmodesmatal connections with adjacent cells are demonstrable during solution of crystal and cellulose in IKI- H_2SO_4 (fig. 94).

In younger leaves the characteristic larger idioblasts are conspicuous immediately beneath both upper and lower epidermis, while the smaller are relatively few in number and may occur near the

fibers of the bundle sheath. In palisade tissue, idioblasts roughly encircle oil glands, and in the spongy tissue, both oil glands and stomata. A semi-quantitative picture of their distribution is given in table 2.

Calcium oxalate may also occur in the form of occasional druses similar in structure and development to those in *Ricinus*.



FIGS. 78, 79.—Mottled leaf. C, chloroplast; IS, intercellular space; ST, starch. Fig. 78, light area, mid-areole, transection of palisade showing large starch grains in small plastids clustered around nucleus (camera lucida). Fig. 79, dark area, near vein, showing larger chloroplasts, parietal in position, containing minute transitory starch grains (camera lucida).

Oil is conspicuous in the repeatedly described oil glands (36, 50) (fig. 17). The schizolysigenous origin of the glands is readily followed in developing leaves. A certain amount of oil is present in other cells in the form of minute droplets.

Hitherto, as far as the authors are aware, suberin has been mentioned in literature about normal leaves only in connection with leaf fall.² Deposition of this substance, however, is not confined to absciss zones and to the time of leaf fall but may be detected throughout all tissues of growing leaves (Figs. 97-110). It becomes increasingly evident with increase in leaf age, and the leathery texture of older leaves depends not only on

thickening of cuticle and strengthening of veins but also on internal suberization. In soft green leaves about half-grown, in which mesophyll differentiation is well advanced, suberin is evident as a film on both outer and inner surfaces of the cell wall. When first detected, both films are at the limit of visibility but stand out distinctly on staining with IKI. The outer film lines the intercellular spaces, the internal surface of the leaf, while the inner resembles a tertiary wall layer within the living cell. Irrigation with H_2SO_4 results in the usual blue coloration and swelling of the cellulose walls and brings the films into clear, though temporary, relief. The film lining the intercellular space darkens, may become granular, wrinkles, and sooner or later is detached from the swelling wall. By the time that the cellulose has completely dissolved, the pellicle is generally fragmented but may still be traced as a dotted line of material insoluble in H_2SO_4 . The intracellular pellicle may behave in the same way but is more tenuous and therefore fragments and disappears more easily. It may adhere partially or completely, however, to the contracted protoplast.

MATURING LEAF

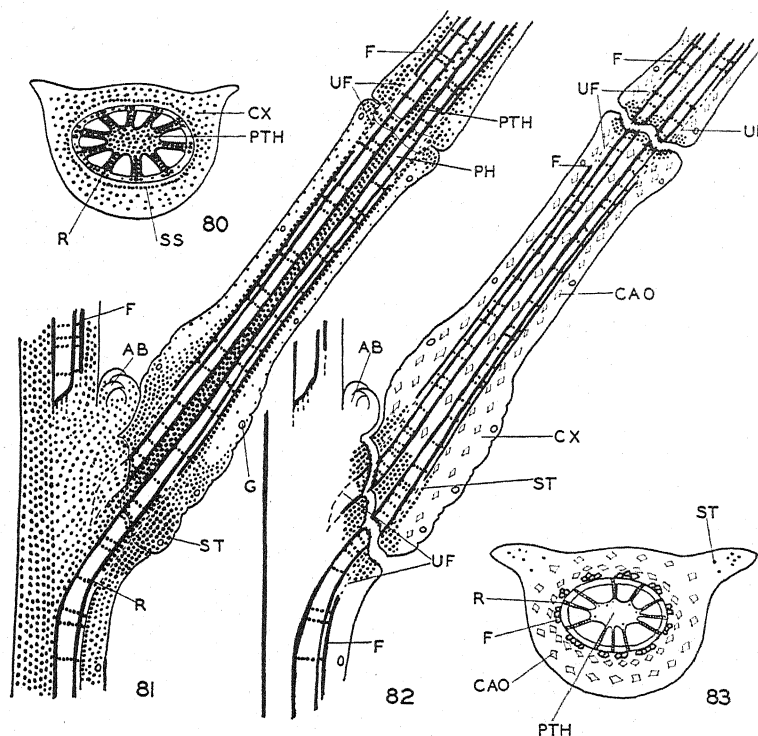
The mature leaf is dark-green in color, firm in texture, and does not wilt readily. Mature leaves differ from younger principally in the following characters: (a) secondary thickening of veins,

² In a letter to Dr. F. M. Turrell from Dr. B. F. Lutman, dated May 30, 1934, the writer stated: "A cursory study of young apple leaf sections, however, has given me the impression that some of the pulp parenchyma cells might be somewhat cutinized." On June 22, 1934, Dr. Lutman continued: "As I wrote you before, in my study of young apple leaves I found from the staining we had used (the Triple, with strong emphasis on the Orange G.) that many of the pulp parenchyma cell walls were apparently cutinized."

(b) change in the starch-calcium oxalate balance; and (c) thickening of cuticle and increase of suberization of internal surface.

SECONDARY THICKENING OF VEINS—As in leaves in general, the cambial zone

and pith, and somewhat less abundant in abscission regions and in the inner cortex of the midrib and major veins. Sooner or later, starch is replaced by calcium oxalate except in vascular rays, outer pith, and the abscission regions.

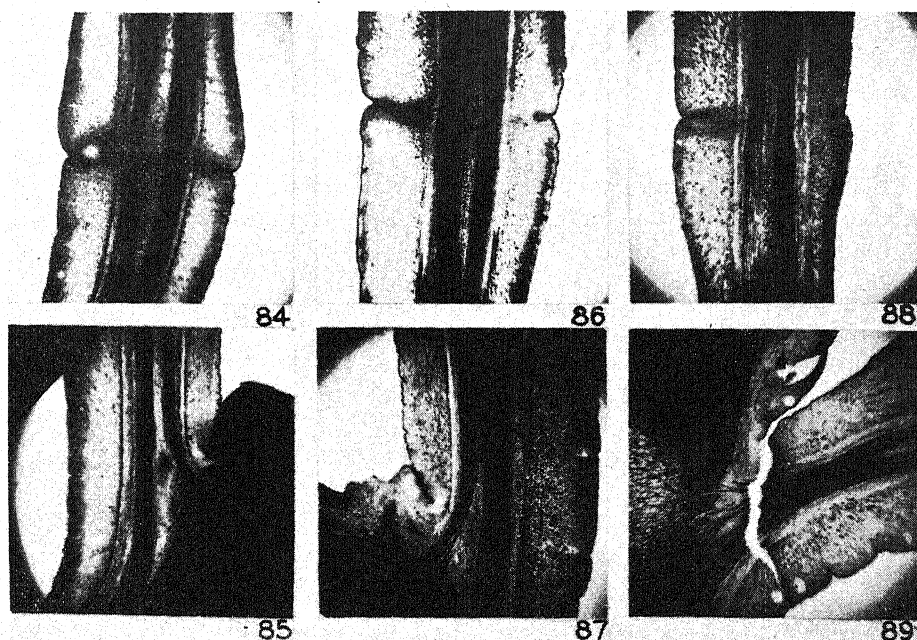


FIGS. 80-83.—Diagrams of starch-calcium oxalate balance. *AB*, axillary bud; *CAO*, calcium oxalate; *CX*, cortex; *F*, fiber; *G*, gland; *PH*, phloem; *PTH*, pith; *R*, ray; *SS*, starch sheath; *ST*, starch; *UF*, unlignified fiber. Fig. 80, transection of base of lamina of mature leaf. Fig. 81, longisection of mature leaf showing starch distribution. Fig. 82, longisection of abscising leaf showing calcium oxalate. Fig. 83, transection of abscising leaf.

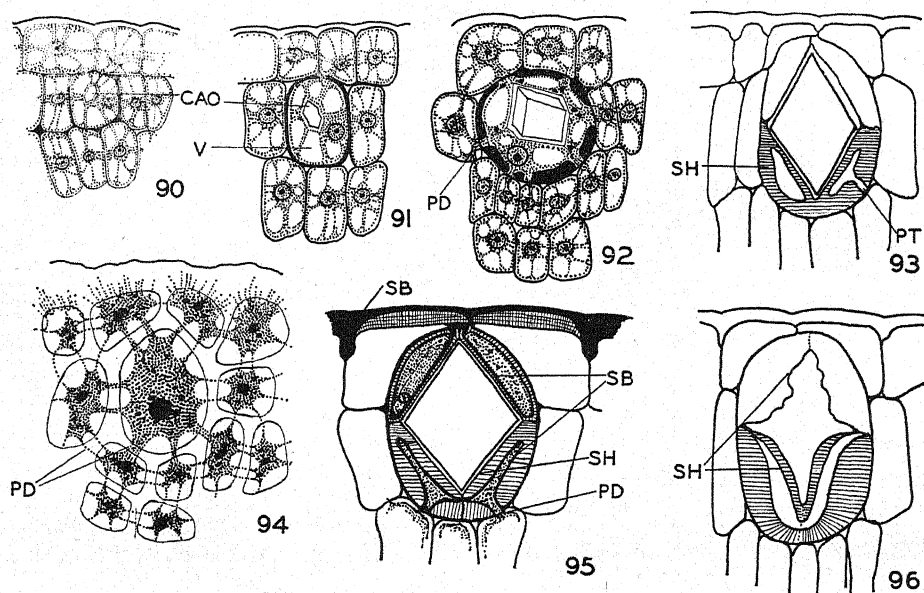
is but weakly defined. In the midrib and major veins of mature leaves, however, typical secondary elements, such as pitted vessels and fibers, supplement the primary spiral elements.

CHANGES IN STARCH-CALCIUM OXALATE BALANCE (figs. 82, 83, 88, 89).—By the time the leaf is mature, reserve starch is relatively abundant in all tissues. It is particularly conspicuous in parenchymatous cells, such as starch sheath, rays,

THICKENING OF CUTICLE AND INCREASE OF SUBERIZATION OF INTERNAL SURFACE.—The epidermis of the older leaves, in contrast to the younger, is heavily cutinized. Flanges of cutin extend inward about one-third of the length of the radial walls. Stratification of the outer wall, wax canals, and pits is identified in the usual swelling reagents. The materials of the stomatal plugs are presumably secreted through the wax



FIGS. 84-89.—Starch distribution in long sections of laminar and nodal abscission zones. Figs. 84, 85, in soft green leaf. Figs. 86, 87, in hard green leaf. Figs. 88, 89, in abscising leaves.



FIGS. 90-96.—Differentiation of calcium oxalate idioblasts. Mid-lamina transections. *CAO*, calcium oxalate; *PD*, plasmodesmata; *PT*, pit; *SB*, suberin; *SH*, sheath; *V*, vacuole. Figs. 90-92, soft green leaves, ± 3.5 cm. long; crystal sheath visible in fig. 92; fig. 90, idioblast size = $12 \times 12 \mu$; fig. 92 = $26 \times 26 \mu$. Figs. 93-96, mature leaves, ± 8 cm. long; fig. 93, idioblast size $95 \times 76 \mu$. Fig. 94, idioblast during treatment with $\text{IKI-H}_2\text{SO}_4$. Fig. 95, suberization of idioblast wall and crystal sheath. Fig. 96, idioblast after solution of crystal in chromic acid.

canals, which terminate on the inner face of the guard cells (figs. 40, 41).

The distinction between internal suberization of older and younger leaves is readily apparent before and after treatment with IKI- H_2SO_4 . Suberin lamellae of intercellular spaces, barely visible in young leaves, are now clearly defined and may be approximately $1\ \mu$ thick, while the intracellular lamellae are somewhat thinner. Suberin lines the smallest intercellular spaces and thence may extend along the middle lamellae until the latter are completely suberized. The result is that, after solution of cellulose in H_2SO_4 , suberin remains in the form of angular fragments—the lining of the small intercellular spaces—or as a more or less complete network if suberization of the middle lamellae has been completed. In addition, there appear fragments of the intracellular lamellae, more or less spherical but usually broken by the swelling of the cellulose walls. Reticular suberization occurs consistently at the bases of both lamina and petiole within ± 2 mm. of the abscission plane. It also extends generally along the midribs and the larger bundle sheaths. As already noted, the starch of the bundle sheath is replaced by calcium oxalate crystals. Complete suberization of sheath and idioblast wall means that the major veins are inclosed within a suberized envelope. Reticular suberization may also extend partially into the mesophyll adjacent to major veins in both lamina and petiole, from leaf base to leaf tip, likewise along the collenchyma of the leaf margin.

Suberization is also evident around the fibers of the pericyclic region and sporadically within the phloem. Presumably in the latter tissue deposition of suberin does not occur sufficiently early to affect transport of reserves (starch) from the lamina (figs. 98–110).

LEAF FALL

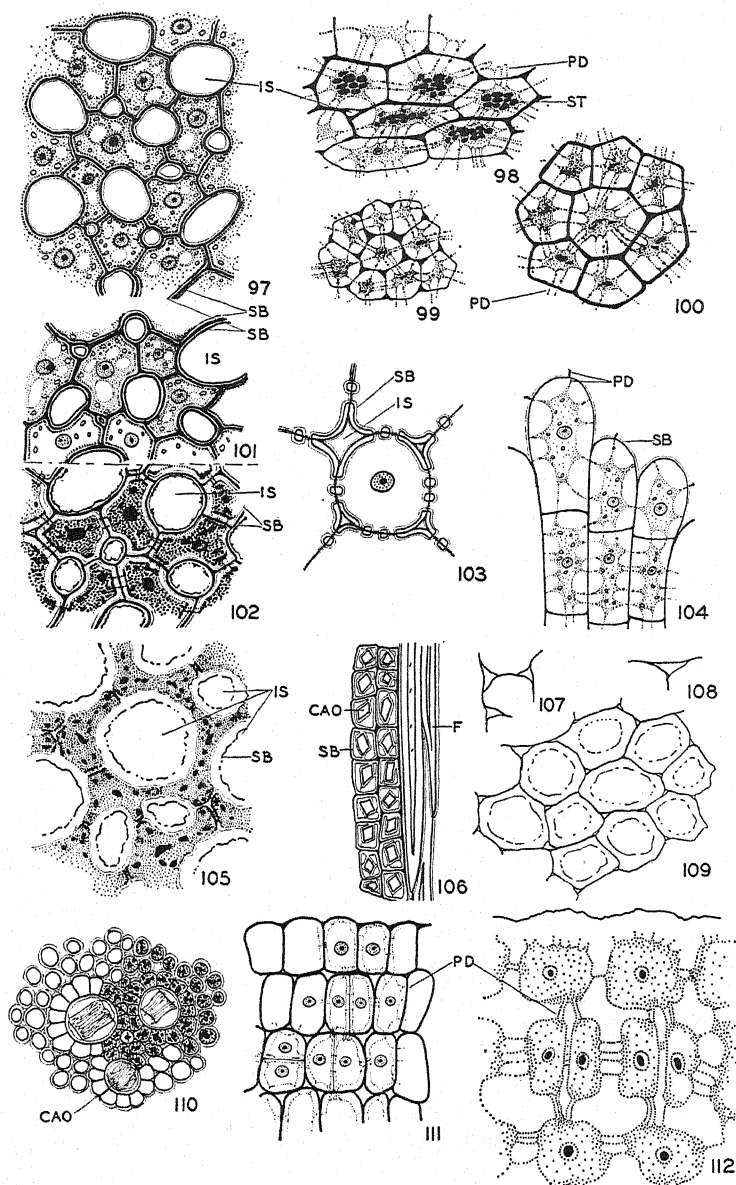
Leaf fall (figs. 113–124) occurs throughout the year but is most active during the seasonal growth flushes. When leaves are about to fall, the laminae and, later, the petioles turn yellow. Laminar abscission precedes nodal; thus, when the laminar absciss zone is fully differentiated, the blade falls at a touch while the petiole remains in place. If the nodal absciss zone is also mature, then the slightest jar is sufficient to cause simultaneous nodal and laminar abscission.

TABLE 2

COMPARISON OF NUMBERS OF CALCIUM OXALATE IDIOBLASTS AND STOMATA SEEN IN SURFACE VIEW OF UPPER AND LOWER EPIDERMIS AND OF OIL GLANDS IN MID-LEAF (CLEARED MATERIAL)

	Upper surface	Lower surface
Idioblasts....	588/sq. mm.	444
Stomata.....	Few on mid-rib	620
Oil glands....	2.9/sq. mm.

LAMINAR ABSCISSION.—The tissues within the laminar-petiole groove include epidermis, collenchyma, abscission parenchyma, and stele. The epidermal cells, except for somewhat heavier cutinization, resemble those of lamina and petiole. The collenchymatous elements, compressed and tapering in outline, fan outward from the constricting groove and continue into the subepidermal marginal collenchyma of lamina and petiole wings (figs. 25, 26). The abscission parenchyma differs from the adjacent cortex of lamina and petiole in the smaller size of the cells and in the lack of all but minute intercellular spaces, but the thickness and pitting of the cell walls are similar in all. The structural weakness of the vascular strand already noted in the young-



FIGS. 97-112.—CAO, calcium oxalate; *F*, fiber; *IS*, intercellular space; *PD*, plasmodesmata; *SB*, suberin; *ST*, starch. Figs. 97-110, suberization of internal surface. Fig. 97, soft green leaf, spongy mesophyll paradermal section; suberin lines intercellular spaces and also inner face of cell wall. Fig. 98, hard green leaf, starch-sheath region, transection; during IKI- H_2SO_4 test, showing plasmodesmata, suberized walls, and swollen starch grains. Figs. 99, 100, hard green leaf, midrib near base of lamina, transections; outer and inner pith during IKI- H_2SO_4 test, showing suberization of walls and plasmodesmata. Figs. 101, 102, hard green leaf, spongy mesophyll, paradermal sections; before and during IKI- H_2SO_4 test. In fig. 102 is seen wrinkling of suberin lamellae in intercellular spaces. Fig. 103, hard green leaf, distal end of petiole, transection; cortex, showing suberization of wall. Fig. 104, abscised leaf, distal end of petiole, longisection; cortex, showing slight suberization of papillate cells. Fig. 105, hard green leaf, spongy mesophyll, transection; during IKI- H_2SO_4 test, showing remains of suberin lamellae. Fig. 106, hard green leaf, midrib, longisection; showing suberization of calcium oxalate idioblasts in bundle sheath. Figs. 107-109, hard green leaf, cortex, transection; after IKI- H_2SO_4 test, showing fragments of suberin network remaining. Fig. 110, hard green leaf, palisade, transection; during IKI- H_2SO_4 test, showing suberin pellicle around palisade cells and transformation of calcium oxalate to calcium sulfate needles. Figs. 111, 112, position of new cell walls. Fig. 111, soft green leaf, 3 cm. long; transection, upper epidermis, and differentiating palisade, showing position of new cell walls in relation to plasmodesmatal areas, during IKI- H_2SO_4 test. Fig. 112, soft green leaf, 3 cm.; similar transection after solution of cellulose walls, showing remaining protoplasts and plasmodesmata.

er leaf is further emphasized at this stage by the partial or complete lack of xylem fibers in the abscission region. The following physiological or structural changes precede or accompany leaf drop: (a) partial replacement of starch by calcium oxalate and disappearance of the remainder (figs. 82, 83, 88, 89); (b) suberization of the basal tissues of the lamina; (c) differential growth of abscission parenchyma; and (d) break of tissues at the separation layer.

Starch accumulates temporarily in all tissues of the mature leaf and is conspicuous throughout the length of the midrib, including the abscission region. Sooner or later starch disappears from the cortex of the midrib, the starch sheath, the central pith, and, to a certain extent, the laminar tissues. It is replaced by solitary ensheathed crystals of calcium oxalate. Prior to leaf drop, the remainder of the starch present in the vascular rays and also in the outer pith disappears entirely except for a few small grains in scattered cells in the suberized basal region of the lamina.

Earlier in this paper the beginnings of intercellular and intracellular suberization of the leaf tissues were described. Suberization increases markedly at the bases of both lamina and petiole. In the lamina all tissues are affected—collenchyma, cortical parenchyma, starch sheath, vascular tissues, and pith. The lignification which frequently accompanies suberization is sparse and appears in a few cells only.

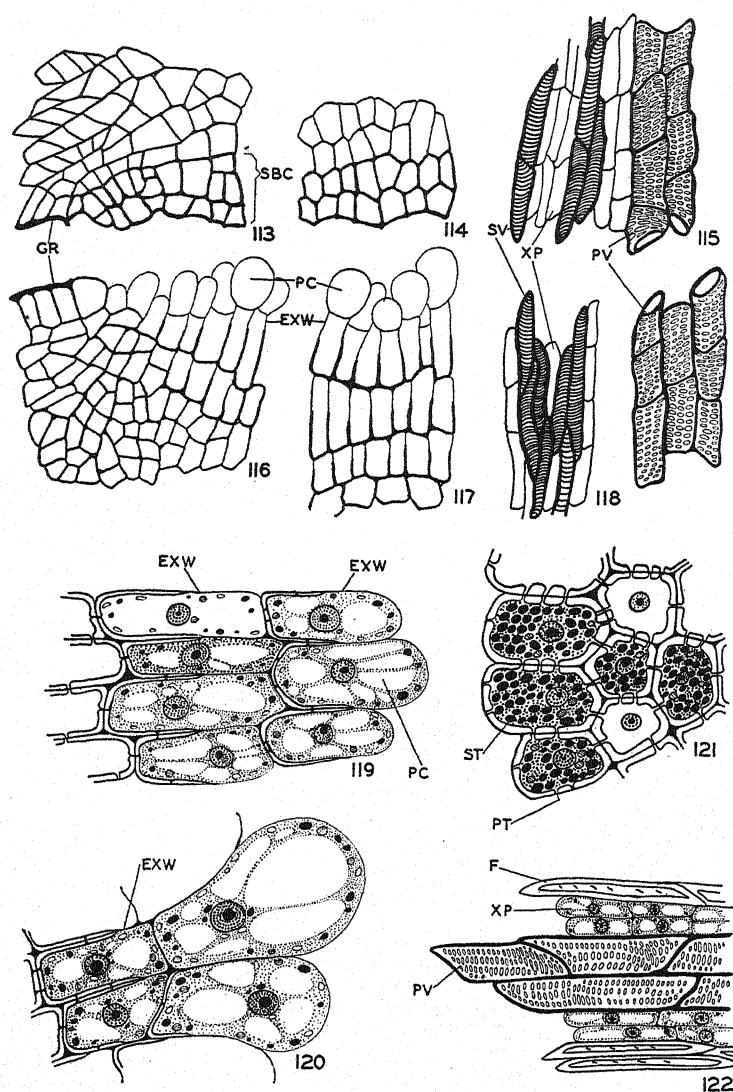
The anatomical changes most significant in actual leaf fall occur in the parenchymatous cells of the abscission zone. Growth occurs in the cells of two or three distal layers of cortex and pith and also to some extent in xylem and phloem parenchyma (figs. 116, 117, 119, 120). As the cell walls extend, these cells may

reach twice or thrice their initial volume. The new cell-wall areas are thin and contrast markedly with the original thickened cell bases. The walls consist of cellulose and pectic substances, but fine suberin films may also be identified (fig. 104). Growth is most active in the middle cortex, and, as the cells expand distally, rupture of the middle lamella occurs between new cellulose and old suberized cell walls. After abscission the enlarged cells round off and form a papillate surface, except for a serrated ring of xylem elements. In the latter the break occurs likewise at the middle lamella (figs. 113, 114, 115, 118, 121, 122). That plasmodesmata are ubiquitous throughout the leaf is apparent, and they are demonstrable in the abscission zones, both laminar and nodal. In the papillate cells minute remnants of protoplasmic strands remain extruded from the free rounded surfaces and indicate the point of previous intercellular connection (fig. 104).

The complex curvature of the abscission faces is clearly seen only after blade abscission. The laminar surface projects roughly as a flattened dome but is incised by a groove ± 0.1 mm. in depth, the line of break of the xylem cylinder. This groove surrounds the slightly projecting core of pith. The petiolar surface forms a shallow cup of corresponding dimensions and is marked by a projecting xylem rim equal in height to the depth of the laminar groove. The central pith is depressed to fit the projecting laminar tip.

NODAL ABCISSION.—The orientation of leaves in relation to light is brought about by curvature of the petiolar base. Hand-sections are, therefore, frequently essential in order to follow the gross distribution of such ergastic substances as starch along the curving median plane from petiole to leaf base and stem.

The nodal abscission zone, like the

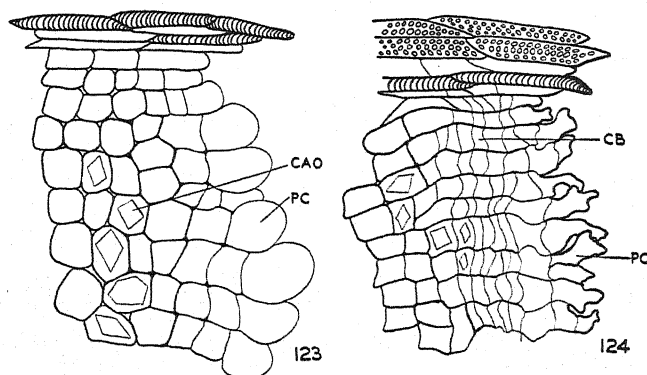


FIGS. 113-122.—Tissues of laminar abscission region after abscission, paradermal sections. *EXW*, external cell wall; *F*, fiber; *GR*, groove; *PC*, papillate cell; *PT*, pit; *PV*, pitted vessel; *SBC*, suberized cell; *ST*, starch; *SV*, spiral vessel; *XP*, xylem parenchyma. Figs. 113-115, base of lamina. Fig. 113, groove and outer cortex, walls of proximal cells thickened and heavily suberized. Fig. 114, middle cortex, cells similar to those in outer cortex. Fig. 115, xylem of midrib, serrate outline resulting from fracture along middle lamella. Figs. 116-118, distal end of petiole opposite laminar base. Fig. 116, groove and outer cortex, surface papillate cells, inner extended cells, distal new cell wall areas thin in contrast to thickened proximal cell bases, faintly suberized. Fig. 117, middle cortex, region of maximum growth, papillate and extended cells. Fig. 118, xylem of midrib, serrate fracture as in laminar base. Figs. 119-120, detail of papillate and underlying cells. Fig. 119, outer cortex. Fig. 120, middle cortex. Fig. 121, base of lamina, middle cortex, detail of thick-walled suberized cells. Fig. 122, distal end of petiole, detail of xylem of midrib.

laminar, is a zone of structural weakness (figs. 27, 123). Pericyclic fibers remain unligified; xylem vessel segments measure one-fourth to one-third the length of these elements in stem and in petiole; and xylem parenchyma is increased at the expense of xylem fibers. The nodal zone differs from the laminar in that, prior to leaf drop, the abscission plane is structurally undefined. In fresh material the plane of rupture is indicated approximately by the differential distribution of

the lamina, all cells are affected, and, as before, lignification is slight and sporadic.

Cell growth occurs in parenchymatous cells of the stem adjacent to the leaf base, i.e., in cortex, vascular parenchyma, and pith. The same contrast in wall thickness is evident as the cells attain the same proportional increase in volume. Growth is most active in the mid-cortex immediately below the median line of the leaf base. Rupture occurs along the middle lamella between thin-walled, en-



FIGS. 123, 124.—Nodal abscission, longisections. *CAO*, calcium oxalate; *CB*, cambium; *PC*, papillate cell. Fig. 123, median leaf trace and cortex in stem, showing papillate cells and serrate outline of broken vein. Fig. 124, leaf scar, ± 72 hours old, showing collapsed papillate cells now suberized and beginning of underlying phellogen.

starch and of intercellular spaces, the position of the shorter vessel segments, and also a slight contrast between the compact chlorenchyma of the stem and the somewhat less compact chlorenchyma of the leaf base.

The physiological and structural changes in the nodal abscission region nevertheless appear similar to those in the laminar region, viz., starch loss, suberization, and differential growth, followed by tissue break. Starch loss is accompanied or not by deposition of calcium oxalate. Intercellular and intracellular suberization is active on the six to ten cell layers at the leaf base. As in

larged cells and suberized leaf-base cells. After abscission, the leaf-scar surface is papillate except for a jagged ring of xylem.

The surface of the leaf scar is slightly concave and is incised by an uneven groove, the maximum depth of which occurs at the breaking point of the median leaf trace. The surface of the leaf base is correspondingly convex and is marked by a projecting xylem rim, uneven in height, the complement of the stem-scar groove. Within 24–36 hours after leaf fall the scar dries out and changes in color from green to buff (fig. 124). In a median section of a scar ± 72 hours old there ap-

pear the shriveled remains of the weakly suberized papillate cells, suberized and somewhat crushed cork cells, and an active cork cambium. The latter continues active as it keeps pace with stem growth.

Discussion

In view of their possible wide application to leaves in general the following statements based on the findings in the Valencia orange leaf merit discussion: (a) plasmodesmata are concerned in the determination of cell shape; (b) plasmodesmata control the orientation of new cell walls in dividing cells; (c) cell expansion with concomitant differential cell-wall growth and suberization of opposing tissues is an important factor in the mechanism of leaf fall; and (e) the hardening of the leaf with age results mainly from progressive suberization of the internal surface. It seems probable that statements *a*, *b*, and *d* will apply to dicotyledons in general, while statement *c* may be shown to be of much wider application than is at present conceded.

CELL SHAPE.—Variation in the shape of meristematic cells is recognized in many papers published in recent years (13, 14, 15, 22). One form, however, which constantly recurs—the tetrakaidekahedron—may be considered the fundamental unit from which all other types in the rind, and also in the leaf, of citrus are derived (35). The same holds true in leaves as different as squash, castor bean, avocado, and sycamore, to mention but a few examined by the authors. Tetrakaidekahedral cells are connected by plasmodesmata, and during expansion to spherical form and throughout later development these intercellular connections are maintained at the surfaces of contact. Plasmodesmata may, therefore, be regarded as relatively fixed points which control future cell development. A spher-

ical cell, comparatively free to expand in all directions, differentiates, as the intercellular spaces increase in size, into an eight-lobed or an eight-armed element. When cell division occurs, the number of cell contacts is temporarily altered to less or more than eight, but during early meristematic growth the original number is sooner or later restored. When growth of the organ as a whole slows down, however, this restoration of contact and plasmodesmatal number does not take place. The result is that mesophyll consists, in the main, of two cell types, eight-lobed or -armed, and six-lobed. Variations in cell form depend on the time of cell division in relation to the growth of the organ as a whole and can be interpreted in terms of division and degree of subsequent expansion of a typical tetrakaidekahedral cell. Specific differences in geometrical form of polyhedral meristem cells produced by the stresses of early growth are transitory and appear to be of minor importance, in comparison with the position of plasmodesmatal connections, in the control of the area and orientation of the curving surfaces of the mature living cells.

WALL ORIENTATION.—In two recent papers on the cytology of wall formation the possible causal factors in wall orientation were discussed (37, 38). In citrus the position of new cell walls follows the general pattern outlined in the above papers, but it appears that physiological, rather than physical, factors are primarily responsible for wall orientation. In the material examined, as already described, the new cell walls are invariably anchored to the wall of the mother cell in plasmodesmatal areas. Deposition of cell walls thus is comparable with the deposition of crystal sheaths seen in *Beloperone* and in *Ficus* (34). The cell wall originates within a cytoplasmic

plate, the phragmosome—the sheath within a cytoplasmic strand. The sheath of the calcium oxalate crystals of the citrus idioblasts is also controlled by the position of plasmodesmata. The factors which activate cellulose deposition within cytoplasmic plates and strands remain to be determined.

ABSCISSION.—The leaf of citrus resembles compound leaves, in that the lamina, which may be considered a terminal leaflet, usually falls before the petiole. Both laminar and nodal abscission zones are, as usual, structurally weak in lack of lignification of pericyclic fibers, reduction of vascular cylinder, comparative increase in vascular parenchyma, and decrease or absence of xylem fibers. In addition, characteristic shortening is evident in primary and secondary vessel segments, their length a fraction, one-third or less, of the length of similar elements in lamina, petiole, and stem.

The anatomy of tissue break resembles that described by LÖWI (16, 17, 18) in *Cinnamomum* and other forms. LEE (12) and PFEIFFER (26, 27) considered this type of abscission to be relatively rare. *Cercis* and *Aristolochia*, however, are presumably similar, since tissue break in the former is attributed to differential growth of cell walls and, in the latter, cell elongation is mentioned in the distal cells of the separation layer.

The question of mechanism of abscission recurs constantly in all papers on leaf fall, whether physiological or anatomical. TISON (41) worked to a great extent with fresh material and therefore stressed "reciprocal pressures" resulting from cell growth as a potent factor in forcing apart leaf base and petiole or rachis and leaflet base in the case of compound leaves. The papillate cells of citrus serve to support this view. In contrast to this, much of the material ex-

amined by LEE (12) was collected at the time of leaf fall but was preserved and examined later. This may be the reason that, although cell elongation was repeatedly described, the idea of reciprocal pressures was not stressed. It seems evident that, whenever cell growth occurs within an abscission zone, cell expansion is one mechanical factor in the final tissue break.

In this connection it is interesting to recall the suggestions of MUSTEL (24) and TREVIRANUS (42), those of the former listed by TISON (41) among the "idéés plus ou moins malheureuses" of earlier workers as a "théorie étonnante." MUSTEL attributed leaf fall to the fact that, since transpiration ceased with leaf age, there was no room in the leaf blade for the ascending sap. The latter therefore pressed against the leaf base and forced its fall. TREVIRANUS noted the heterogeneity of tissues in the abscission zone and suggested that this condition prevented sap circulation, interrupted the vital connection between petiole and node, and resulted in tissue rupture. In view of the suberization of leaf tissues described in this paper in citrus and other leaves—most intensive at the leaf base but general throughout the mesophyll—it may be questioned whether the earlier theories are as completely farfetched as they appeared to TISON. Physiological experiment with aging leaves is necessary to clarify the effect of progressive suberization on such fundamental functions as cell permeability, transpiration, and transport of water and food and on the process of leaf fall.

In discussing the causal factors in cork formation, PRIESTLY pointed out the interconnection between food supply, the presence of air, a blocking surface formed by deposition of suberin, and the origin of a phellogen (31). The pattern in citrus

is significant. Suberin is deposited adjacent to parenchymatous tissues with abundant intercellular spaces, and food is present in the form of starch. The phellogen appears after the suberization of the surface of the leaf scar. The physicochemical reasons for intensive suberin formation remain to be determined.

INTERNAL SUBERIZATION.—Suberin occurs consistently in the abscission zones of higher plants, but its deposition was entirely unexpected in the general tissues of lamina and petiole. Suberin appears not only in citrus but in leaves as diverse as squash, avocado, castor bean, broad bean, and sycamore. It is identified in the young, soft-growing leaf as a film at the limit of visibility, while in the old, hard, mature leaf it is a clearly defined pellicle. In the old, hard, yellowing leaves of sycamore, suberization is sufficiently strong to prevent the tissue disintegration which follows irrigation with H_2SO_4 . During the progress of the $IKI-H_2SO_4$ test the light-blue color characteristic of dissolving cellulose is masked and replaced by deep purple in the practically unswollen cell walls. At the end of this drastic treatment the suberized mesophyll of the leaf still persists intact.

In discussing the penetration of spray oil into citrus leaves, ROHRBAUGH (33) pointed out the difficulty of postulating capillarity as the primary force concerned. "... capillarity depends upon the cohesion of the liquid to the surface of the capillaries. If the surface of the cell wall is covered with a lipid film or some other surface to which the oil could cohere, then capillarity might well be the explanation of the movement of the oil into the tissue." A suberized internal surface may satisfy this condition. Spray oil may pass through the epidermis via stomata and also wax canals and may thereafter spread more or less freely over

the suberized internal surfaces. It is significant that oil accumulation is most marked in regions of heavy suberization—the cells surrounding the midrib and also along the leaf margin.

Summary

1. The development of the leaf in the Valencia orange is considered in four stages: (a) resting bud and meristematic primordia; (b) expanding leaves; (c) maturing leaves; and (d) leaf fall. Stages a-c parallel similar phases in stem differentiation. The differentiation of vascular and other tissues is described.

2. Cell shape is considered in detail in spongy and in palisade parenchyma. Two principal cell types are common to both, the eight-armed or eight-lobed cell and the six-armed or six-lobed.

3. In the spongy mesophyll, eight-armed cells occur at vein level, in mid-areole, the region of largest intercellular spaces in which cell division first ceases. The eight-armed cell may be derived from a tetrakaidekahedral meristematic cell in plasmodesmatal connection with eight others.

4. In the lower mesophyll and along the margins of the areoles, cell division continues later than in mid-mesophyll and here six-armed cells differentiate. The six-armed cell may be derived from a tetrakaidekahedral element which has undergone comparatively late cell division.

5. In the palisade parenchyma the counterparts of the eight- and six-armed cells are eight- and six-contact cells, in which the lobes through which the plasmodesmata pass are reduced to a minimum.

6. The above cell types occur in all dicotyledonous leaves so far examined by the authors.

7. Plasmodesmata may be regarded as relatively fixed points on the cell surface and therefore are of importance in controlling cell shape and wall area. Cell shape depends also on the time of cell division in relation to the growth of the organ as a whole.

8. Plasmodesmata are directly concerned in the orientation of new cell walls. In all cells examined, new walls are anchored to the wall of the mother cell in plasmodesmatal areas.

9. The position of the sheath of calcium oxalate crystals is, as in the idioblasts of *Beloperone* and *Ficus*, controlled by plasmodesmatal connections. Cytoplasmic strands in crystal sheath formation resemble, in their function of wall deposition, cytoplasmic plates—the phragmosomes of normal cell division.

10. Leaf abscission occurs between lamina and petiole and also at the leaf base. The mechanism is the same in both. Papillate cells produced by distal elongation of cells of the abscission zones expand outward against heavily suberized tissue at the bases of lamina and of petiole. Tissue break occurs at the junction of the thin-walled papillate cells and the opposing suberized elements.

11. Suberin deposition occurs not only in abscission regions but throughout the mesophyll of the leaf. In half-grown leaves it appears in the intercellular

spaces and also within the cell as a film at the limit of visibility. In mature and old leaves suberin lines the internal surface and impregnates the middle lamella to a greater or lesser extent and also forms a tertiary lamella on the walls of the inner surfaces of all cells.

12. Progressive suberization appears to be a general phenomenon in the aging of leaves, since suberin deposition occurs in squash, castor bean, avocado, and sycamore leaves. In sycamore (*Platanus racemosa*) suberization is sufficiently heavy to prevent the usual cell-wall swelling and tissue disintegration which result from treatment of leaf sections with $\text{IKI-H}_2\text{SO}_4$. The suberized mesophyll persists as intact network.

13. Suberization of the internal surface presumably facilitates the distribution by capillarity of spray oil which may enter the leaf.

14. The development and structure of guard cells and of oil glands is similar to that seen in the fruit rind.

15. The wax canals in the outer wall of the epidermal cells resemble those of the fruit rind, and through them the surface wax and the material of the stomatal plugs is secreted.

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LITERATURE CITED

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ORIGIN AND DEVELOPMENTAL MORPHOLOGY OF ROOT NODULES OF *PISUM SATIVUM*

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Introduction

The morphology of the root nodules of leguminous plants is of interest for several reasons. The leguminous nodule is a structure unlike any produced by other plants. Although formed only after bacterial infection, the nodule is not an irregular mass of tissue, such as composes certain bacterial galls, but has differentiated tissues in a definite arrangement. The structure of nodules is also related to certain physiological problems. The nodule is one product of the interaction between invading rhizobia and root cells. Experiments using "tagged" nitrogen have demonstrated clearly that neither rhizobia nor leguminous plants alone can fix atmospheric nitrogen but that nitrogen fixation results from an appropriate combination of the two (6). The mechanism of this fixation remains unknown but is evidently an interaction between host and bacterial cells. The reaction is localized in those cells of the nodule that are actually infected with bacteria.

The present investigation was undertaken to attempt to supplement our knowledge of the developmental morphology of root nodules on leguminous

plants, with special reference to the tissues proliferating and the differentiation of the nodule. Pea (*Pisum sativum* L.) was selected for study because, although it has been used in many laboratory and field experiments on nitrogen fixation, reports of the development and structure of its nodules are incomplete.

Review of literature

The anatomy of the nodules of many leguminous plants has been studied by numerous investigators, and the resulting literature has been surveyed by several writers (12, 16, 27, 28). The earliest record of gross observations of nodules is found in drawings made by FUCHS and DALECHAMPS in the sixteenth century (12). In 1679 MALPIGHI explained the swellings on roots of *Vicia faba* as insect galls, although he could find no insect larvae in them (12). Nineteenth-century investigators noted the general regions visibly differentiated within root nodules but proposed various erroneous explanations of the nature and cause of the tubercles until near the end of the century (16). A series of experiments then proved the true etiology. FRANK found no nodules on plants grown in sterilized soil; WARD proved that infection of the roots resulted in nodules; BEIJERINCK isolated in pure culture the infecting bac-

¹ Investigation carried on at the University of Wisconsin, where the author was a Wisconsin Alumni Research Foundation Research Assistant in the Department of Botany.

teria; and in 1890 PRAZMOWSKI published an account of nodule development in pea that established the facts that nodules result from the infection of the roots with rhizobia and that the "infection threads" visible in root hairs and cortical cells are zoöglöeal strands imbedding the bacteria (12, 16). Among the investigators of this period, BEIJERINCK and several others considered the nodules as pericyclic in origin, but a larger number, including PRAZMOWSKI, thought that nodule tissues were derived from cells of the root cortex (16).

Investigations since 1900 have revealed considerable variation among different genera in both shape and internal structure of nodules. Certain of the differences in structure have been used as bases for classifying nodules into types (11, 18, 20), but these groupings do not agree, nor do they correlate well, with either taxonomic or cross-inoculation groupings of leguminous plants. Thus, the differences in nodule structure considered by these authors do not serve as a wholly satisfactory basis for nodule classification.

In spite of some differences in the structure of mature tubercles, the course of nodule development in most herbaceous plants studied is fundamentally the same. The similarities and the few major differences in the pattern of nodule development are demonstrated in papers that have appeared since 1900, concerning the nodule development of many cultivated herbaceous legumes. These include studies of nodules of the following plants: alfalfa, *Medicago sativa* L. (2, 21, 23); bean, *Phaseolus vulgaris* L. (8, 16), and runner bean, *P. multiflorus* Willd. (11); broad bean, *Vicia faba* L. (4, 11); bur clover, *Medicago denticulata* Willd. (19); clover, *Trifolium* spp. (7, 11, 15, 29, 30); cowpea, *Vigna sinensis* Endl. (2);

lupine, *Lupinus albus* L. (8, 18), *L. mutabilis* Sweet and *L. perennis* L. (18), and a hybrid (11); pea (7, 8, 11, 15, 29, 30, 31); peanut, *Arachis hypogaea* L. (1, 2, 14); soybean, *Glycine max* Merr. (2, 7, 11); sweet clover, *Melilotus alba* Desr. (2, 29); sweet pea, *Lathyrus odoratus* L. (29); and vetch, *Vicia sativa* L. (8) and *V. villosa* Roth. (2, 15). Only a few of these papers give detailed accounts of nodule anatomy—those of THORNTON (21) on alfalfa, MCCOY (16) on bean, PEIRCE (19) on bur clover, ALLEN and ALLEN (1) on peanut, BIEBERDORF (2) on soybean, and WIPF and COOPER (31) on early stages in pea.

In most herbaceous legumes rhizobia invade the young root through root hairs, and the first evidence of infection is a curvature at the tip of the root hair (2, 4, 16, 19, 21, 31). This curling is brought about by some substance secreted by the bacteria, as is evidenced by the fact that cell-free extracts of bacterial cultures will induce curling (17, 22). The bacteria congregate and/or multiply at the surface of the hair, usually near the bent tip (19, 22). In some manner, they penetrate the cell wall and begin to multiply inside the root hair. There is no visible rupture of the wall at the point of entry (19), and the rhizobia have not been shown to be capable of dissolving the wall materials, cellulose, calcium pectate, and hemicellulose (17). The characteristic curvature of infected root hairs must have been brought about by unequal growth; this continued growth of the cell after initial infection is evidence that the root-hair cell is relatively intact (19). Inside the root hair, the bacteria multiply and secrete a characteristic gum. The bacteria and surrounding gum form the zoöglöeal infection strand, which may branch as it passes through the host cell (16, 21). The host cells, first

in the epidermis and then in the cortex, gradually wall off this strand, producing a cellulose sheath whose presence, according to MCCOY (17), probably gave rise to early reports that the infection strand was a fungal hypha (9). Proper staining reveals the bacteria imbedded in the zoöglöeal strand (4, 8, 16). The fact that the cellulose wall is discontinuous at intercellular spaces shows that it is laid down by the host cells rather than by the infection strand (17).

The infection strand grows inward from the tip of the root hair to the inner wall of the epidermal cell, a process requiring about 2 days in soybean (2), and then penetrates the cortical cells. The depth of penetration of the cortex varies somewhat with the plant being invaded. In bur clover the strand grows nearly straight toward the central cylinder of the root and penetrates as far as the layer of cortical cells next to the endodermis (19). In alfalfa, also, the infection strands penetrate the cortex to its inner layers but do not enter the endodermis (21). In bean, in which the nodules are not efficient in nitrogen fixation, the infection threads penetrate only the outer layers of the cortex (16). In soybean, the infection strand penetrates from three to five layers of the cortical parenchyma cells (2), and in pea it reaches the inner layers of the cortex (31).

The development of the nodule begins with division of host cells in the immediate vicinity of the end of the infection thread. In alfalfa, nuclei of cells adjacent to the infection strand swell, and active nuclear and cell divisions begin (21). These divisions extend to a distance of two or three cells from those in the cortex already reached by the infection thread, and some divisions occur in the endodermis and pericycle. PEIRCE (19) found early stages in the development of the

nodule "just like a young lateral root"; but WIPF and COOPER (31) showed that, in pea, proliferation begins definitely in the cortex. WIPF and COOPER found that many of the cortical cells stimulated to active division by the invading rhizobia are disomatic; they noted earlier (30) that the $4n$ chromosome number is characteristic of nodule tissue. In alfalfa (21) branches of the infection strand penetrate the proliferating cortical cells, and division of the cells soon ceases except in a region toward the root surface that remains meristematic; the bacteria are released from the strand into the host-cell cytoplasm by the formation of blister-like swellings on the infection strand. The rhizobia multiply in the host-cell cytoplasm, and, after the cell is fairly well filled with bacteria, division of the host cell ceases. In soybean (2) the bacteria are released similarly and also by breaks in the infection strands that occur with division of the host cells; in bean (16) the bacteria are spread chiefly by division of the infected cortical cells. Cytological changes in the infected host cells are most conspicuous in the nucleus, which becomes large and has prominent chromatic bodies, and in the cytoplasmic inclusions and mitochondria (1, 16).

Mature legume nodules have an infected or bacteroid region with a meristematic zone either apical to or surrounding the bacteroid tissue. Outside the infected cells is a zone of noninfected cells—the nodule cortex—through which run vascular strands that connect with the stele of the root. The nodule is still surrounded by part of the root cortex in which some divisions and considerable stretching must have occurred, but the root epidermis is usually broken (12). An endodermal layer has been noted between the root cortex and the nodule (4, 8, 11). Old nodules show disintegration

beginning in the older part of the bacteroid region, and some old nodules slough off before the end of the season (2, 16).

There are some exceptions to the history of nodule development as given above. There is evidence that rhizobia may enter roots through broken cortical cells or ordinary epidermal cells (2, 16), and experimentally they may be introduced by pricking or stroking the root with a needle previously dipped in the bacterial culture (8, 19). The peanut is an exception in that invasion seems to occur always through cortical cells ruptured by the emergence of a lateral root. The peanut also differs from other leguminous plants studied in that its nodule is definitely pericyclic, rather than cortical, in origin and that it breaks through the cortex of the root (1).

Material and methods

Three varieties of the cultivated pea were used: Canada field pea, Alaska field pea, and Perfection garden pea. The plants were grown in the greenhouse, three or four to an ordinary tumbler, in gravel that had been washed thoroughly and screened to yield only pebbles 2-8 mm. in diameter. Mineral nutrients were supplied by a Crone's salts solution (5), minus nitrogen except for the uninoculated control plants which were supplied with potassium nitrate. This method of culture gave root aeration as good as or better than that in sand or soil and eliminated the difficulty encountered in sectioning when small particles adhere to the roots.

To insure infection and effective nodulation, the seeds were sterilized, soaked overnight in sterile distilled water, and inoculated. The method for sterilizing was as follows: peas were placed in a

sterile suction flask and a 1:1000 solution of mercuric chloride was added; this solution was kept under vacuum for 5 minutes, and then the seeds were rinsed twice in sterile distilled water under vacuum; next, a solution of "B.K." powder (calcium hypochlorite) to give 3% available chlorine was added and the mixture kept under vacuum for 10 minutes; finally, the seeds were rinsed in eight changes of sterile, distilled water. The sterilized and soaked seeds were inoculated by dipping them into a sterile-water suspension of a culture of *Rhizobium leguminosarum* Frank, U.W. strain # 302 or # 329, effective on peas. The inoculated seeds were planted in the tumblers of gravel, which had been sterilized by autoclaving at 15 pounds pressure for 4 hours or longer. Sterilized Crone's salts solution was supplied once a week, and sterile distilled water was added whenever the solution level dropped too low.

When plants were harvested, the whole root systems were washed out of the gravel. Some were examined fresh; others were cut into pieces for fixation, in most cases under reduced pressure, in Karpechenko's or Belling's modification of Navashin's solution or in Flemming's medium solution. They were dehydrated, then cleared and infiltrated by the chloroform or the cedar-oil method, and imbedded in paraffin. Material sectioned at a thickness of 5-10 μ was stained in Heidenhain's iron-alum haematoxylin, Flemming's triple stain, or safranin-Delafield's haematoxylin. One lot of material was cleared in *eau de Javelle* as for a whole mount, stained in basic fuchsin, imbedded, and sectioned.

Plants were grown at several seasons: spring, early fall, and late fall, 1942; late winter, and summer, 1943; and summer, 1944. Fixations were made of plants ranging in age from 1 to 8 weeks, in order

to include a series of stages in nodule development.

Observations and discussion

The three varieties may be distinguished by their general habit of growth. The variety Canada produces tall slender plants with small leaflets and long internodes; Alaska is larger and has somewhat stouter stems and larger leaflets, but the internodes are about as long as those of Canada peas; Perfection has short internodes, stout stems, and large leaflets. The anatomy of roots and nodules is very similar in the three varieties, and, unless differences are noted, statements made apply to all three varieties. No distinction is made between plants inoculated with the two different strains of *R. leguminosarum*, since they showed no visible differences in nodule development or structure.

Nodules on the roots of the varieties studied develop to macroscopic size within 2 weeks after the seeds begin to germinate. Their rate of development varies with external conditions, but visible nodules usually are found on plants that have one or two fully expanded pinnate leaves. Light is the environmental factor of greatest influence on nodule development, although temperature has some effect. Unless artificial illumination is used, nodules may be entirely absent from plants grown in the greenhouse during winter months. The importance of light in determining the carbohydrate-nitrogen relation of the plant and, therefore, nodule initiation and development has been discussed in detail by WILSON (28). Nodules first appear on the upper portion of the primary root, always below the hypocotyl, becoming visible about the time that secondary roots are protruding beyond the cortex of the primary root. For a week or two, as the

plant grows, additional nodules develop on the lower part of the primary root and on the upper portion of many of the secondary roots. A plant several weeks old has a well-developed root system with conspicuous tubercles (fig. 1). The peripheral portions of the root system do not bear nodules following inoculation with an effective strain, such as strains # 302 and # 339 used in these experi-

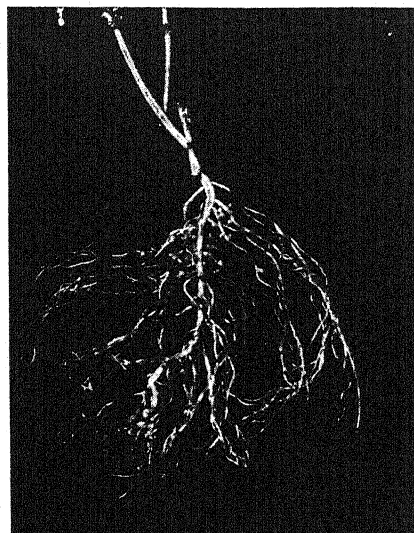


FIG. 1.—Root system of Canada pea showing effective nodulation. $\times \frac{1}{2}$.

ments. The changed carbohydrate-nitrogen relation in such plants probably accounts for this distribution of nodules (12).

Since one aim of this investigation was to determine which tissues proliferate to form the nodule, observations were made of normal root structure and of the origin of lateral roots, to give a basis for comparison with developing tubercles. VAN TIEGHEM and DOULIOT (24, 25) described both the structure of the root and the origin of its branches, and HAYWARD (13) pictured and described the mature primary root. The present observations

agree, in general, with these authors but include additional information, especially with regard to maturation.

The root is composed of the following tissues: an epidermis that is not lost even in quite old roots; a cortical parenchyma consisting of six to ten cell layers in the larger roots and four to five cell layers in smaller branch roots; an endodermis with distinct Casparian bands that are occasionally less prominent over the protoxylem points; a pericycle composed of large parenchymatous cells, a single layer opposite the phloem, and two or three layers opposite the xylem; phloem, consisting of sieve tubes and companion cells, fibers, and parenchyma; xylem that is triarch in most of the primary and larger secondary roots, that is not infrequently tetrarch in these roots, and that is diarch in the smaller branch roots; and a noncontinuous cambium that lays down a limited amount of secondary tissue, mostly xylem, between the primary xylem and phloem.

In the course of root development and, to some extent, in different parts of the same root, there is a correlation between the stage of development of a nodule and the degree of maturation of the part of the root on which it is located. The rate of growth and the condition of the infected root seem to be more important than actual time intervals in determining the stage of development of the nodule. Only young nodules are found in the region of maturation, but the mature region has fully developed nodules. Nodules on the old region of the root—with some secondary tissues in the stele—show disintegration in their bacteroid portions. The tissues in each of these regions of a primary or large secondary root, such as would bear nodules, will be described in some detail because of their relation to the developing tubercles.

In the maturation region, in which root hairs are well developed, the protoxylem and part of the metaxylem become differentiated, and Casparian strips are laid down in the endodermis (figs. 2, 3, 7). The xylem element abutting the pericycle appears in longitudinal sections to be a small annular vessel. The next element is usually much larger and is a spiral to scalariform vessel. The first metaxylem vessels have scalariform thickenings. The remainder of the xylem is composed of undifferentiated parenchymatous cells at this level of the root. The first phloem is visible on a radius between the xylem points and lies next to the single layer of pericyclic cells. Whether this earliest phloem is parenchymatous or is made up of sieve tubes is difficult to determine. In roots of Canada peas, the phloem fibers are beginning to differentiate at this level, and the earlier phloem cells are already somewhat crushed between the pericycle and the developing fibers. These partly crushed cells appear to be parenchymatous. It is possible, however, that these cells might be sieve tubes. The recognition of sieve tubes in transverse sections of Canada pea roots is possible in the later-formed phloem centripetal to the group of fibers. Here, the shape of the cells and associated companion cells and the thickenings of the longitudinal walls (the *nacré walls*) are distinctive. Lack of companion cells and loss of the *nacré wall* thickenings—both of which may occur in protophloem (10)—would account for a parenchymatous appearance of early sieve tubes. Probably because of the small size of phloem elements in Canada pea, sieve plates are not readily distinguishable in transverse section, although visible in longitudinal section (fig. 4). In longitudinal section the sieve tubes show conspicuous dumbbell-shaped slime

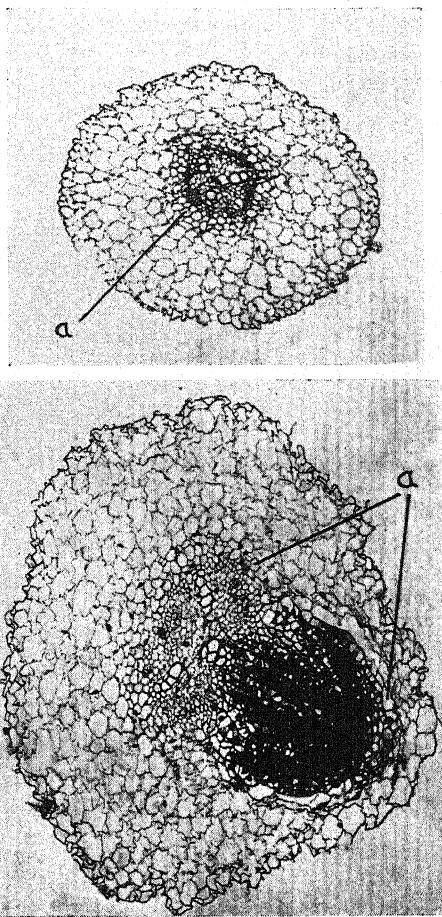
bodies that stain bright red with safranin. In Perfection peas the cells are larger, and the sieve plates, especially in the metaphloem, are more readily visible. The sieve plates are only slightly, if at all, oblique and are easily recognizable if stained with safranin and examined under high magnification (figs. 5, 6). Like those of the metaphloem in the variety Canada, the sieve tubes are also recognizable by their associated companion cells and thickened longitudinal walls. Figure 5 shows two sieve tubes associated with a few small parenchyma cells, the group evidently constituting the proto-phloem. In some cases a single sieve tube, separated from the pericycle by a small parenchyma cell, occupies a position midway between the two xylem arms. The sieve tubes of the protophloem are smaller than those of the metaphloem in the same plant but are similar in having companion cells and visible sieve plates. In the variety Perfection the phloem fibers differentiate a little later than in the variety Canada. Figures 5 and 6 show several sieve tubes, chiefly metaphloem. Most of the group of thin-walled cells between them will mature as fibers. Cells in the protophloem and some of the cells formed centripetal to the fibers are crushed as the fibers mature.

In the mature region the metaxylem is differentiated to form a protosteles. Frequently there is a single large vessel in the center of the root (figs. 12, 13, 14). The later metaxylem elements are scalariform and pitted vessels. The phloem fibers form conspicuous patches alternating with the xylem arms and adjacent to or nearly adjacent to the pericycle.

The cambial layer differentiates in arcs from parenchymatous cells between phloem and xylem. Its limited activity results in the addition of xylem vessels and some scalariform tracheids cen-

tripetally and a small amount of phloem centrifugally. The diameter of the stele is increased only slightly, and the cambial layer remains discontinuous (fig. 16).

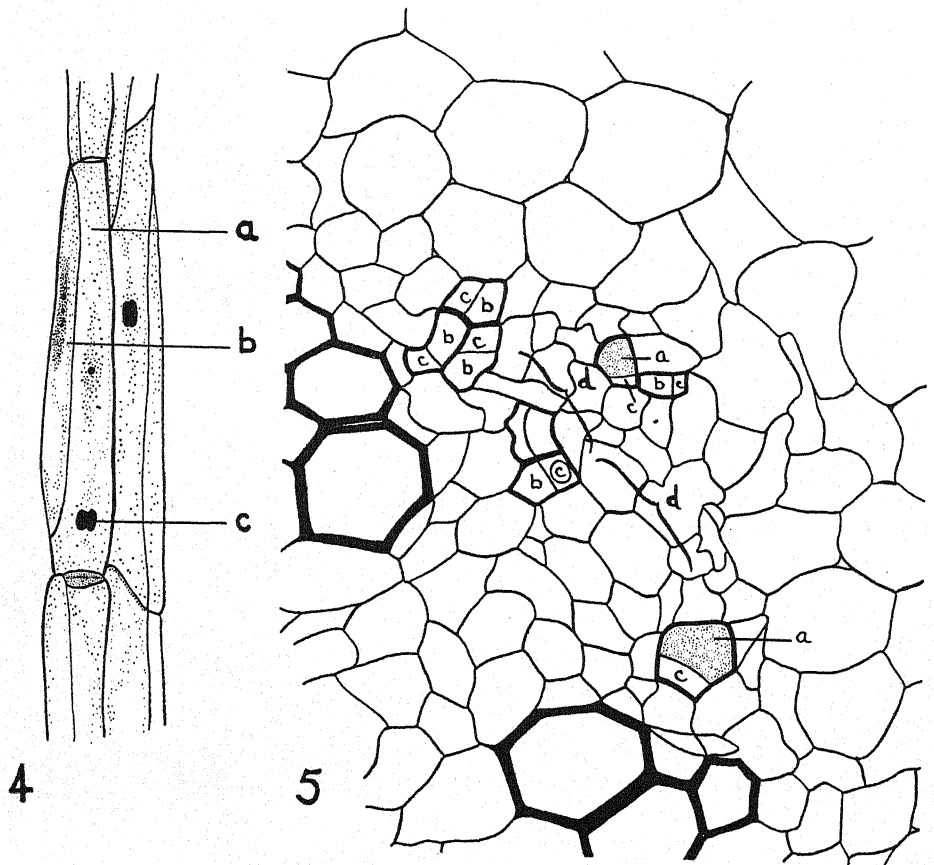
Secondary roots arise in the pericycle. The first indication of the origin of a secondary root in pea is the tangential division of pericyclic cells opposite one of the protoxylem points (13). BOTTUM (3) traced the origin of the secondary root in *Melilotus* to the division of a single peri-



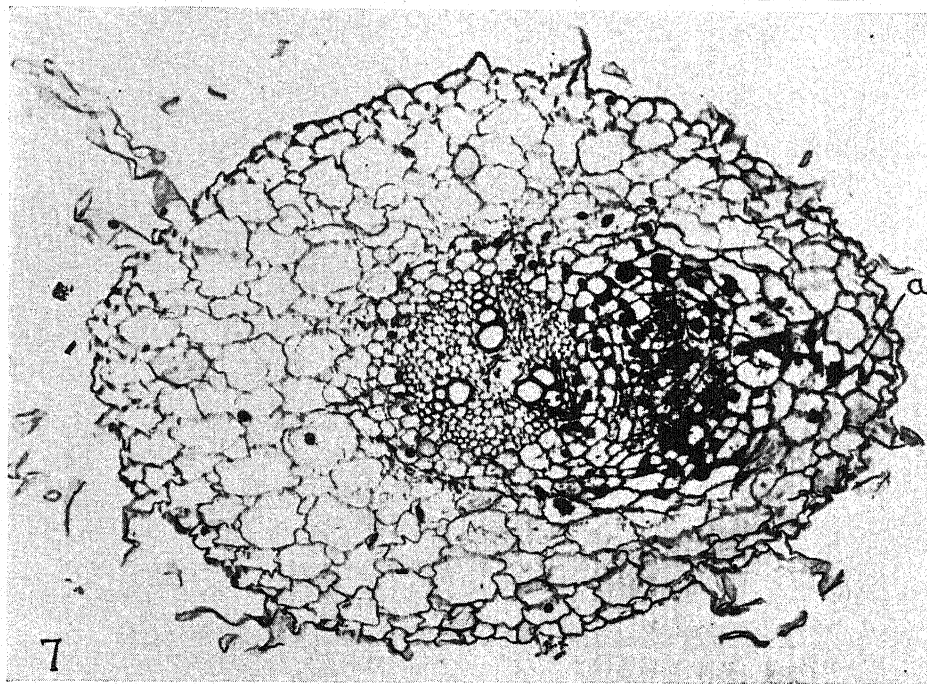
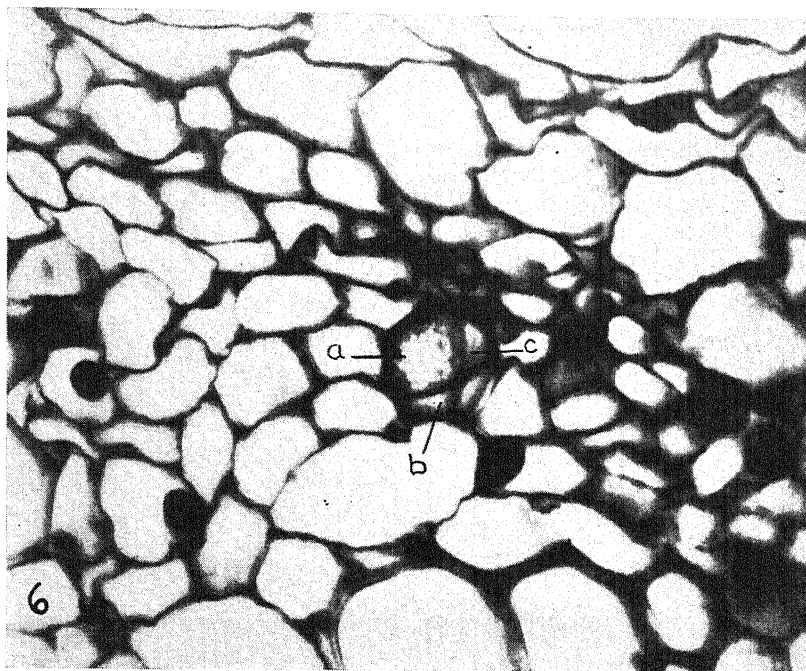
FIGS. 2, 3.—Cross sections of pea roots. $\times 100$. Fig. 2 (above), through region of maturation of secondary root; variety Perfection; a, endodermis. Fig. 3 (below), at level of emerging lateral root; variety Canada; a, root endodermis.

cyclic cell lying between protoxylem and the endodermis. In pea, which has more than one layer of pericyclic cells opposite a xylem arm, the divisions apparently occur first in the outermost pericyclic cells but soon involve the two or three layers of the pericycle. As the secondary root grows outward, the endodermis remains for a time as a layer surrounding it. The endodermal cells opposite the center of the root tip divide radially as the primordium develops, but no roots observed showed tangential division to give several layers of endodermis outside the new root cap, such as was described by VAN

TIEGHEM and DOULIOT (24). Only a few of the newly formed endodermal cells show Casparian thickenings. The endodermal cells along the sides of the secondary root do not divide so much as do those opposite the center of the root tip but stretch and finally rupture as the root elongates (fig. 3). The tissues of the secondary root follow this general description of the primary root. The tip has a meristem of the open type described by HAYWARD (13) for the primary root. Fairly early in the development of the branch root, the plerome, which will develop into the stelar region, can be dis-



FIGS. 4, 5.—Fig. 4, sieve tubes and companion cells from mature root of Canada pea, longitudinal section; a, sieve tube; b, companion cell; c, slime body. Fig. 5, part of stele of root pictured in fig. 2; sieve plates in face view at a, sieve tubes at b, and companion cells at c; d, immature fibers. $\times 1100$.



FIGS. 6, 7.—Fig. 6, part of stele of root pictured in figs. 2 and 5; sieve plate in face view at *a*; companion cells at *b* and *c*, not forming vertical row, but at different levels. $\times 1300$. Fig. 7, cross section of root of Canada pea at level of very young developing nodule; rhizobial infection strand at *a*. $\times 250$.

tinguished from the surrounding periblem (fig. 3). Earliest differentiation of vascular tissues in this region of the branch root proceeds outward from the phloem and xylem of the primary root, with which the basal plerome cells are in contact from the start. The secondary root develops an endodermis that is joined to the primary root endodermis across the base of the cortical region of the branch root.

Cross sections of pea roots, at the level of developing nodules, showed in every case that bacterial invasion occurred through a root hair (figs. 7, 9). These observations agree with those of WIPF and COOPER (31). They found that some infection strands continued growth through almost the entire cortex without apparent effect on the cortical cells. In most cases, however, they noted division figures, many of which showed the disomatic chromosome number, in cortical cells near the tip of the infection strand. In the present investigation, every infection strand found had produced some effect on the cortical cells, although in one case divisions of the cortical cells had been very few and had ceased before a typical nodule was formed.

Early stages in the proliferation of root tissues in the formation of nodules were observed in the varieties Canada and Perfection slightly less than 2 weeks after the inoculated seeds were planted—about 10 days after germination. In both varieties the infection strands penetrate about half the distance from the epidermis to the cortex. In either primary or secondary roots this may include three to five layers of cortical cells, depending upon the size of the root. Those cortical cells which lie in an arc around the end of the zoöglöeal thread, between the tip of the strand and the endodermis, soon are stimulated to divide. These

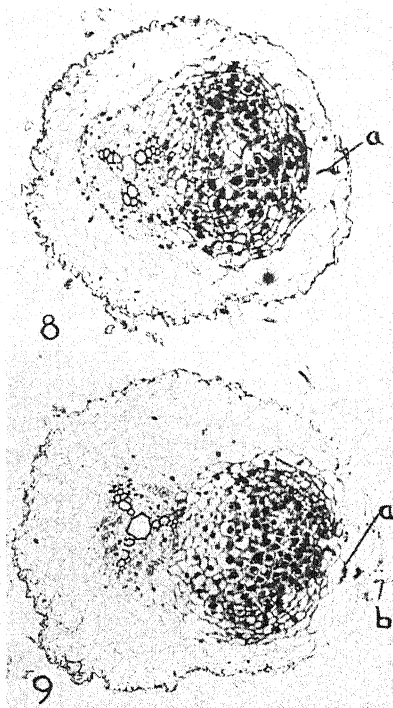
early divisions are not followed immediately by cell enlargement, so that the young nodule appears for a time as a mass of small, densely protoplasmic cells surrounding the tip of the rhizobial zoöglöea (fig. 7). At levels at which vascular strands will develop, tangential divisions of the pericyclic cells adjacent to the region of cortical proliferation soon occur, and, in a few cases, the endodermal cells between had definitely divided tangentially. In one case both daughter cells had Casparian bands. This is not usually true, and, where divisions have occurred in the endodermis, that tissue is difficult to follow in a young nodule. At other levels the root endodermis does not lose its identity. The cells that divide first in response to the presence of the rhizobia are outside the endodermis and, therefore, definitely cortical. This agrees with observations made by WIPF and COOPER (31). Therefore, the nodule does not originate in the same tissue as the secondary root, as was considered to be the case by DANGEARD (8) and by others.

The nodule enlarges at first through continued tangential divisions of cortical cells about a center approximately at the tip of the infection strand. Further enlargement of the tubercle is brought about by an increase in cell size as well as by cell division. The characteristic regions of the nodule become delimited as it increases in size. Mitoses gradually cease in most cells in the portion of the young nodule near the stele but continue toward the periphery of the root, so that a meristematic region becomes delimited apically in the nodule (figs. 12, 14). Those cells in which division ceases in the basal portion of the nodule enlarge and mature as parenchymatous cells. The cells which will give rise to the vascular strands remain meristematic. The latter

cells divide radially, forming the provascular strands (figs. 12, 13).

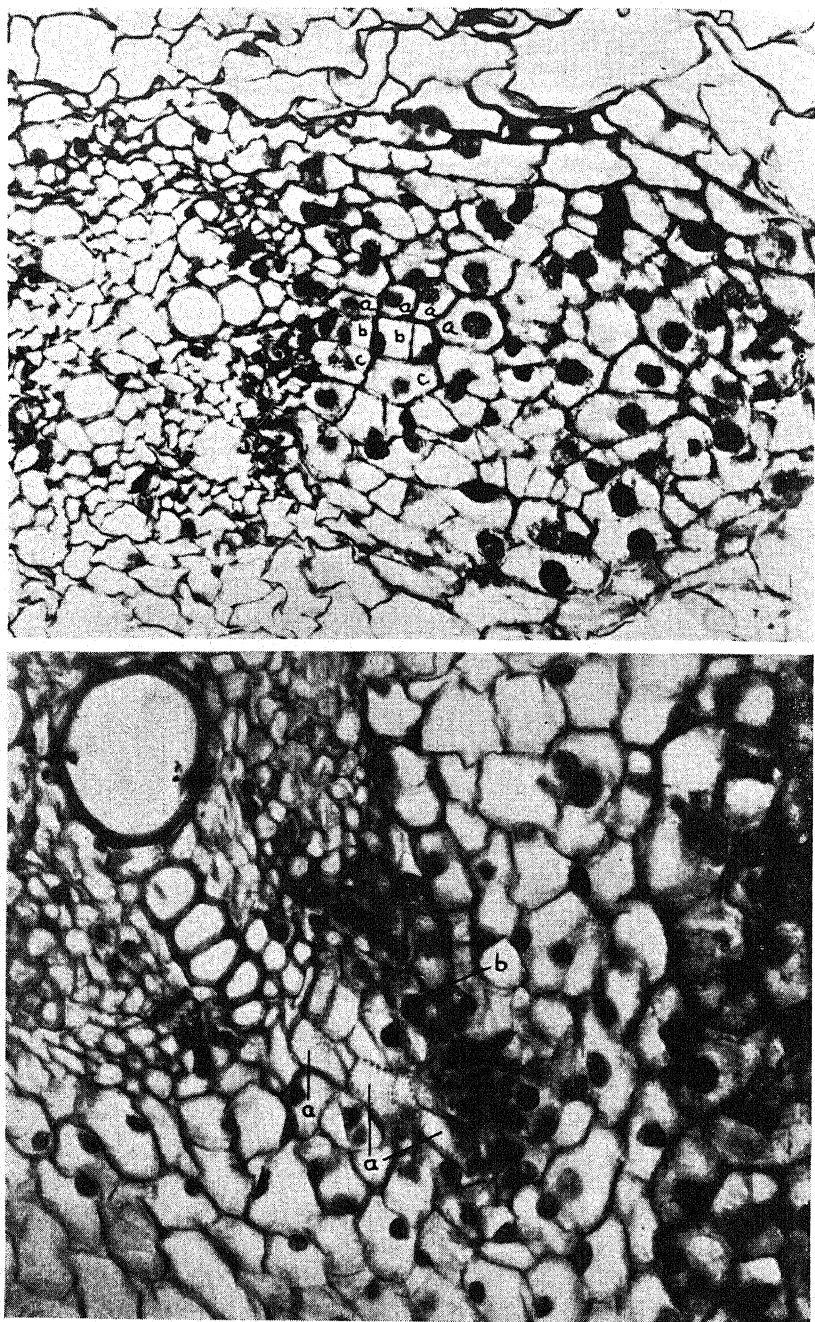
The bacteroid region next becomes visibly distinct. As cells of the young tubercle enlarge, branches of the infection thread penetrate many of them. Bacteria are released within some cells that contain infection strands, evidently by a partial dissolving of the gum imbedding the rhizobia. This occurs in a portion of the strand not inclosed by a cellulose wall, as was observed also by THORNTON (21). The bacteria multiply in the cytoplasm of the host cells, the cells gradually assuming the appearance typical of the infected cells of the bacteroid region (fig. 20). The nucleus of each infected cell becomes much enlarged and contains a number of large, dark-staining bodies that, from a study of intermediate stages in their formation, appear to be aggregations of nuclear chromatin. A cell from the bacteroid region of the nodule has a large central vacuole, and the cytoplasm is filled with bacteria of various shapes—rods, clubs, and X- or Y-shaped branched forms. Occasionally, a few starch grains are found in the peripheral layer of the cytoplasm of infected cells. Branches of the rhizobial zoöglöea continue to intrude into many of the new cells laid down by the nodule meristem, but in no case were infection strands found in the outermost layers of the meristematic region. Figures 18, 19, and 20 show groups of cells selected at intervals of about ten cell layers from the nodule meristem. The cells of the first group are from the meristem proper, those of the second have infection strands in them, and the cells in the third group are from the edge of the bacteroid region. Older cells than those pictured in figure 20 have bacteria much more closely packed in the cytoplasm, and the rhizobia in such cells stain less

distinctly. Probably because of the cellulose walls formed around them, the zoöglöeal threads maintain their identity, and their branches are recognizable throughout the bacteroid area, especially in nodules on Canada peas. Most cells



FIGS. 8, 9.—Transverse sections of roots of Canada pea at level of young developing nodules, before differentiation of vascular tissues in nodules; *a*, infection threads in cells of outer cortical parenchyma; *b*, infection strand in root hair. $\times 100$.

containing bacteria obviously have become infected by means of these strands, but the branches are not sufficiently numerous to account for all the infected cells. In a few cases, division figures were observed in cells already containing in the cytoplasm a few rhizobia that would become distributed at cytokinesis to the two daughter cells. In no case was a typical bacteroid cell—one with numerous bacteria already present—observed in a



FIGS. 10, 11.—Transverse sections of pea roots. $\times 450$. Fig. 10 (*above*), variety Perfection; part of stele and young nodule at level of differentiation of one nodular bundle; *a*, *b*, *c*, radial rows of cells with nuclei in prophase stages of first radial divisions to form base of provascular strand. Fig. 11 (*below*), variety Canada; first maturation of vascular tissues at base of nodular bundle; *a*, scalariform tracheids; *b*, radial division in phloem.

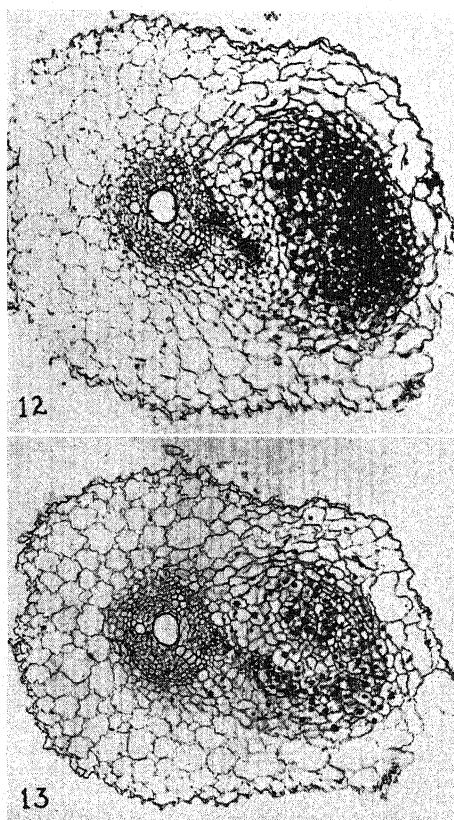
division stage. Some cells lying among those that are infected contain no bacteria. These cells have smaller nuclei than adjacent infected cells, and their cytoplasm frequently contains starch grains (fig. 20).

By the time that the nodule meristem and the bacteroid region are clearly recognizable, the nodule has enlarged so that it protrudes well beyond the original line of the root periphery. The root epidermis is broken, but cells of the cortical parenchyma divide and stretch considerably so that they remain as the outermost layer of the nodule (figs. 12-17). The nodule never emerges from the original root cortex as does a secondary root.

Within a week after initiation, the nodule has about the same diameter as the root, and the tissues characteristic of the mature nodule become differentiated in its basal portion. Surrounding the bacteroid region, except on its side adjacent to the nodule meristem, is a nodule cortex of uninfected parenchymatous cells. These cells, distinguishable from the outer, root cortex by their smaller size, are part of the nodule proper, because they are derived from the general meristematic region of the nodule. Each of the vascular bundles differentiated in this nodular cortex is surrounded by a typical endodermal layer with Casparian bands; this layer will be referred to as the "vascular endodermis" or "bundle endodermis." In addition, the outermost layer of the uninfected cortical parenchyma of the nodule develops Casparian bands, thus forming an endodermal layer between the nodule proper and the outer cortex made up of stretched cortical cells of the root. This outer endodermal layer will be referred to as the "nodule endodermis." The junction of these endodermal layers of the nodule with the endodermis of the root at the base of the

mature nodule is of interest. The root endodermis loses its identity only at the levels at which the two main vascular bundles of the nodule become differentiated; this provides further evidence that, with the exception of the base of each vascular strand, the tissues of the nodule are cortical in origin (figs. 23, 24).

In pea, the nodule becomes more or less cylindric in shape through the action of the apically localized nodule meristem. All the tissues of the nodule—the bacteroid region, the cortex, the vascular strands, and the nodule endodermis—



FIGS. 12, 13.—Transverse sections of root of Canada pea. $\times 100$. Fig. 12, with nodule vascular strand pictured at higher magnification in fig. 11. Fig. 13, nearer periphery of nodule shown in figs. 11 and 12, showing continuity of provascular strand to apical meristem of young nodule.

differentiate acropetally as new cells are laid down by the meristem. The meristem continues to function long after the oldest bacteroid cells have begun to disintegrate, but the nodule eventually (after about 2 months, in this material) becomes a hollow shell filled with a mass of bacteria and disintegrated host cells (figs. 16, 17).

The vascular system of the mature nodule has a fairly regular pattern. Two main vascular strands differentiate in the base of the nodule (figs. 22, 25). These bundles branch dichotomously one or more times, giving a total of from six to

ten strands. Each main bundle usually branches twice, so that the number of vascular strands most frequently visible in a subapical transverse section of the nodule is eight (fig. 26). The origin of the two strands which connect to the root stele varies according to the location of the nodule. If the infection strand penetrates into the cortex almost directly opposite the phloem of the stele—that is, midway between two xylem arms—the two bundles usually originate one adjacent to each of the two xylem arms and at nearly the same horizontal level. If, as is more frequently the case, the infec-

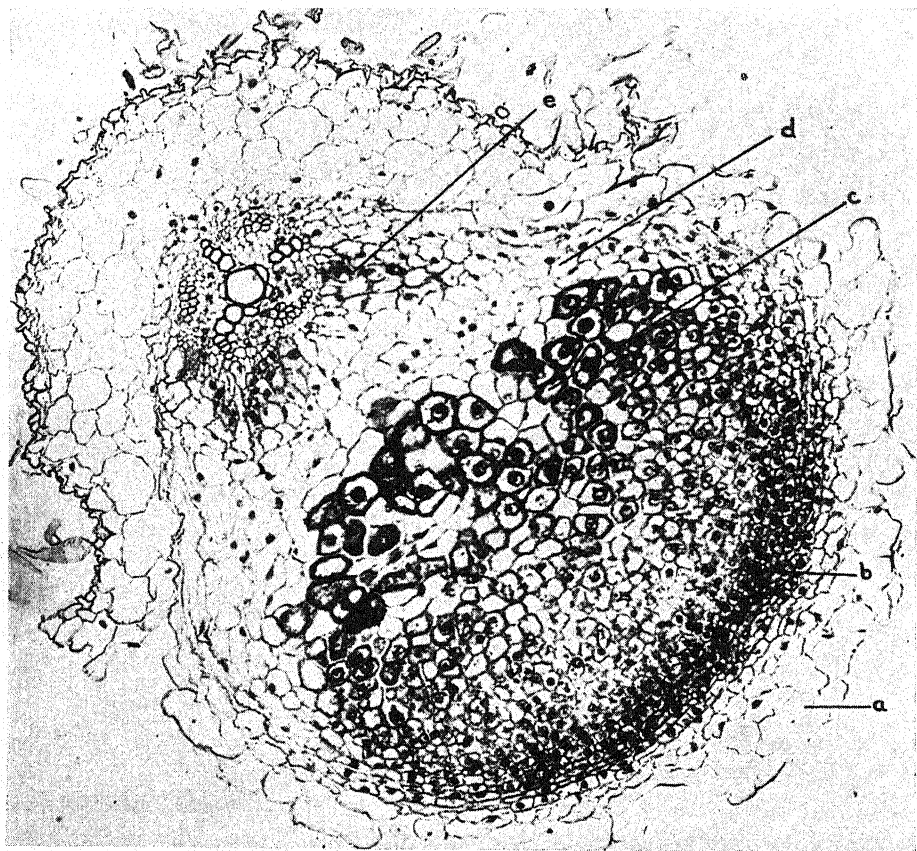
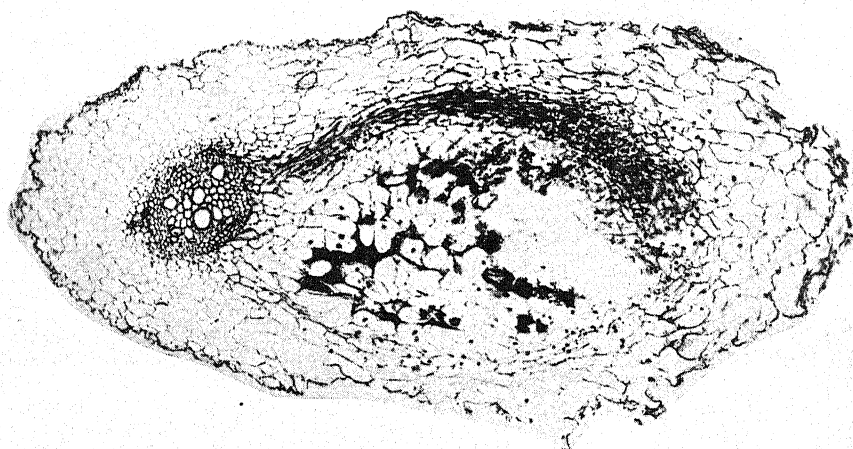
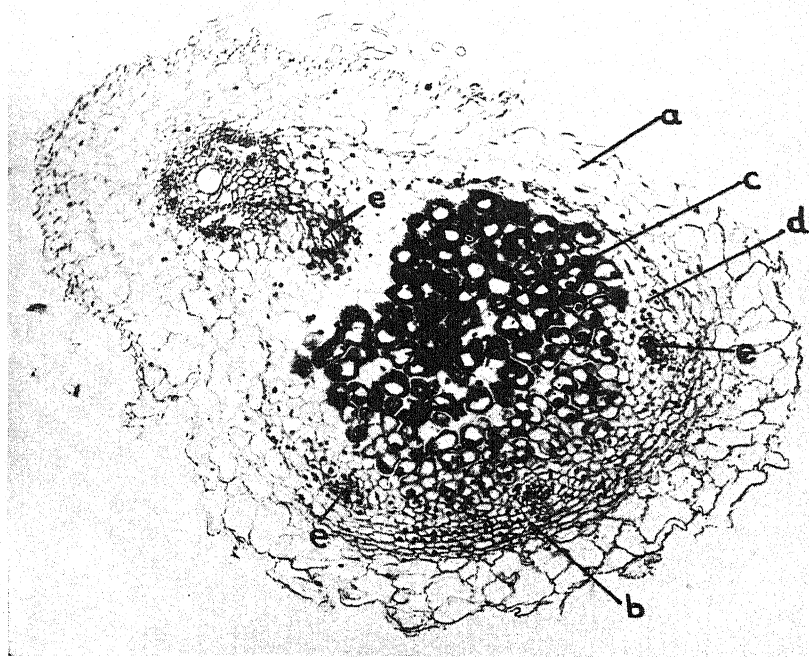


FIG. 14.—Transverse section of root of Canada pea; nodule in which all characteristic regions are differentiated; *a*, outer root cortex; *b*, nodule meristem; *c*, bacteroid region; *d*, nodule cortex; *e*, vascular strand. $\times 200$.

tion thread penetrates to a point nearer to one xylem arm than to another, both nodular bundles are connected with that xylem arm, one above the other. In one exceptional nodule of the first type, there were four main strands, two connecting,

one above the other, to each of two adjacent xylem arms. If the two main strands are one above the other, the first two pairs of dichotomous branches lie in a horizontal position. If the two main strands attach to two different

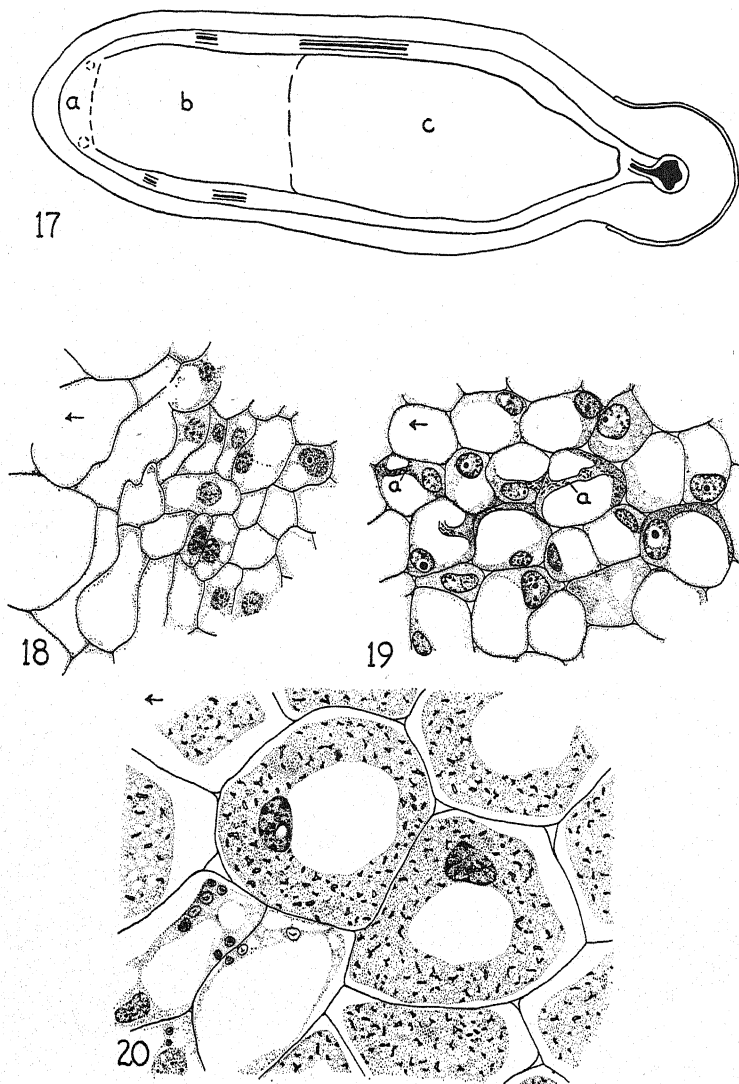


FIGS. 15, 16.—Transverse section of roots of Canada pea. Fig. 15, nodule slightly larger than that in fig. 14. Regions as in fig. 14. $\times 100$. Fig. 16, old nodule with bacteroid region disintegrating. $\times 70$.

xylem arms, the first branching occurs in more or less vertical planes. The branches of the vascular strands are continuous to the persistent meristem, and in transverse section as many groups of provascular cells as there are branches are visible very near the tip of the nodule.

There is no evidence of anastomosing or joining of these strands at the apex of the tubercle.

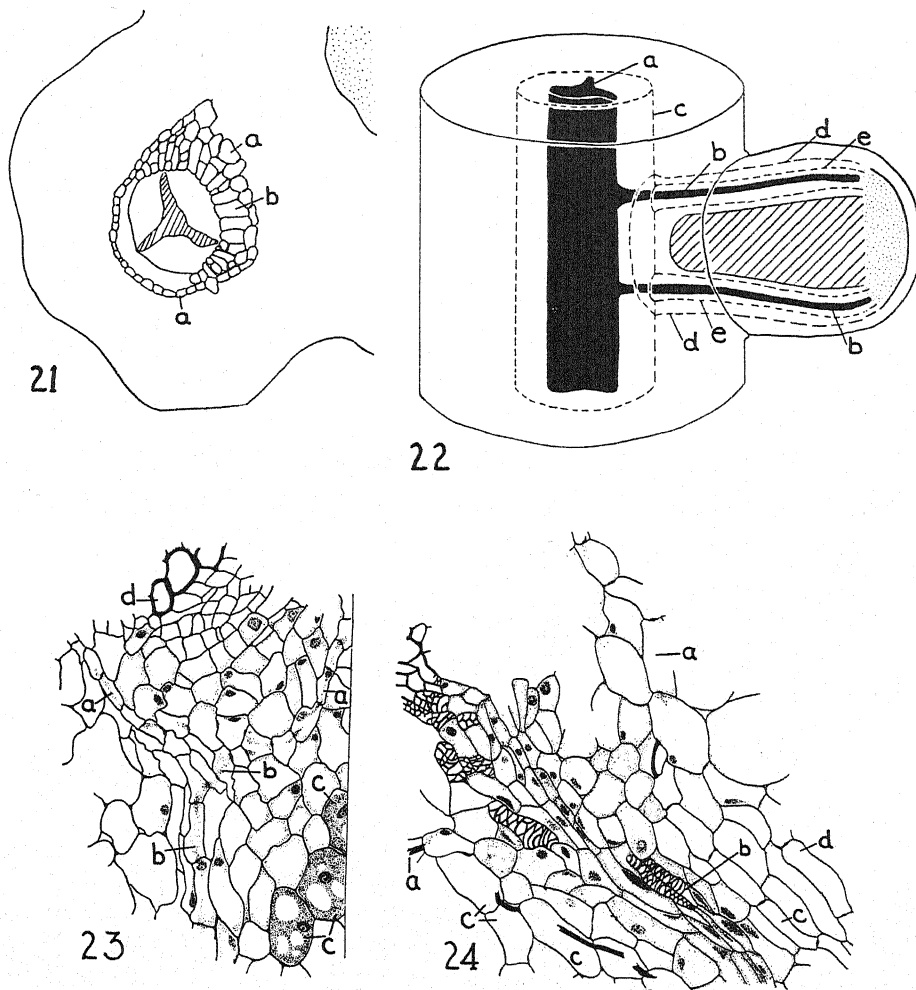
In a study of the ontogeny of the vascular system, the first indications of the origin of nodular bundles are the tangential divisions of cells in the pericycle



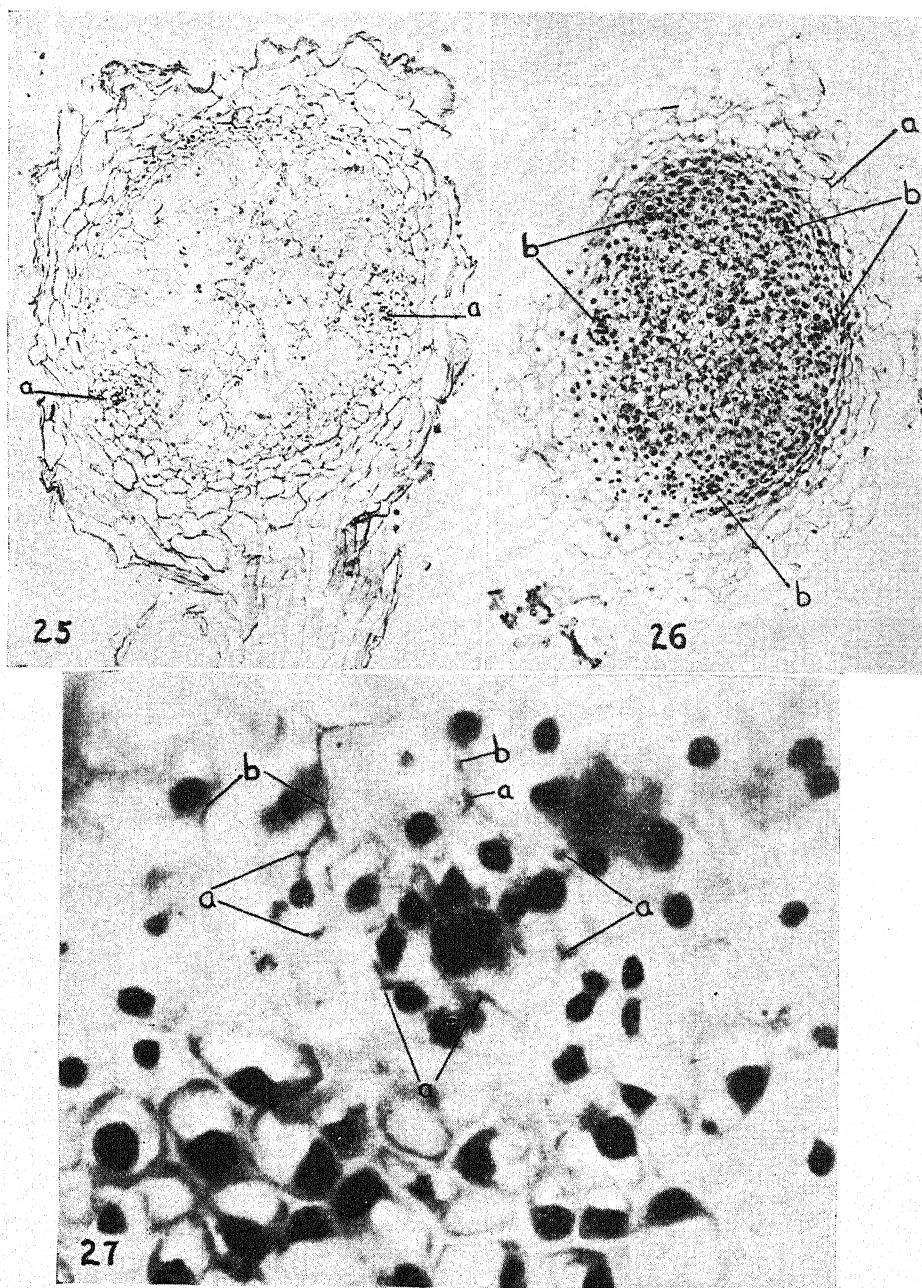
FIGS. 17-20.—Fig. 17, diagram of transverse section of root of Canada pea showing old nodule, cylindric in shape; *a*, nodule meristem; *b*, bacteroid region; *c*, disintegrating old bacteroid region. $\times 40$. Figs. 18, 19, 20, groups of cells selected at intervals of about ten cell layers from meristem of mature nodule of Alaska pea; fig. 18, from meristem; fig. 19, intrusion of infection strands, *a*; fig. 20, from bacteroid region. $\times 550$.

and endodermis of the root. Serial transverse sections of roots bearing young nodules show that only at the levels at which the two main vascular strands of the nodule differentiate is there active division of pericyclic cells or any division of endodermal cells. This observation is

confirmed by study of both transverse and longitudinal sections of roots bearing slightly older nodules (figs. 21, 22). At levels of the nodule above and below the two vascular bundles, the pericycle opposite the root phloem consists of a single layer of cells that have undergone some



FIGS. 21-24.—Fig. 21, semidiagrammatic representation of cross section of root of Canada pea at level near nodular bundle; *a*, root endodermis; *b*, pericycle. $\times 75$. Fig. 22, diagrammatic representation of root and nodule, showing two main nodular bundles; *a*, root xylem; *b*, xylem of nodular bundle; *c*, root endodermis; *d*, nodule endodermis; *e*, nodular bundle endodermis. $\times 75$. Figs. 23, 24, part of transverse sections through base of nodule of Alaska pea. $\times 250$. Fig. 23, junction of nodule endodermis with root endodermis; *a*, root endodermis; *b*, nodule endodermis; *c*, infected cells of nodule; *d*, protoxylem of root. Fig. 24, junction of nodule endodermis and nodule vascular endodermis with root endodermis; *a*, root endodermis; *b*, nodular bundle; *c*, nodule vascular endodermis; *d*, nodule endodermis.



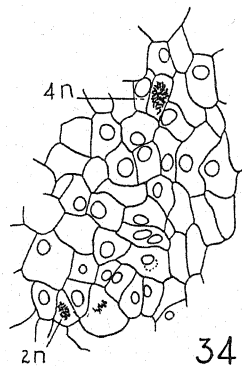
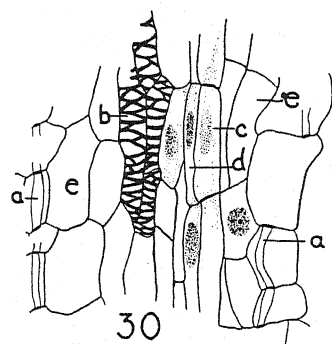
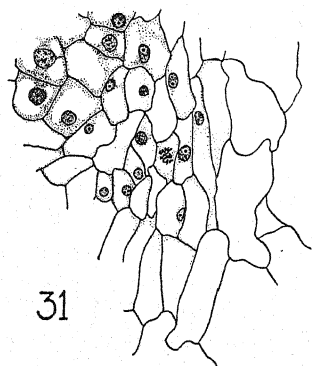
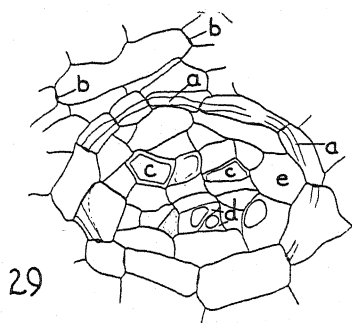
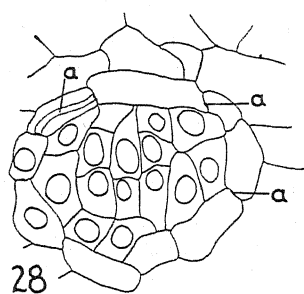
FIGS. 25-27.—Fig. 25, tangential section of root of Canada pea through base of nodule, showing two main nodular bundles in cross section, *a*. $\times 100$. Fig. 26, cross section near apex of nodule shown in fig. 25; *a*, nodule outer cortex; *b*, provascular strands in cross section. $\times 100$. Fig. 27, cross section of provascular strand at level a little farther from tip than fig. 26, showing Casparian bands in bundle endodermis, *a*, and in nodule endodermis, *b*; vascular tissues of bundle not differentiated. $\times 450$.

hypertrophy; opposite the xylem, the pericycle consists of the usual two or three layers of cells, which show some radial elongation.

The second stage in the development of the vascular strands of the nodule is a marked radial elongation of rows of cells derived from the tangential divisions of the pericycle opposite the xylem and phloem of the root. Then, almost simultaneously, radial divisions occur in two to several rows of cells, one continuous from the side of the xylem arm to the endodermis, another continuous from the sieve tubes lying nearest one side of the xylem arm, and sometimes a row continuous with the phloem abutting the other side of the xylem arm (fig. 10). There follow very closely similar divisions of the endodermal derivatives adjacent to these rows. Then, simultaneous radial divisions of cells in the basal region of the nodule form provascular strands that are continuous to the apical meristem of the young nodule (figs. 12, 13).

Differentiation of the vascular tissues is acropetal. By the time that the basal parenchyma cells of the nodule are enlarged and definite provascular strands can be seen between the root stele and the nodule meristem, maturation is almost complete in the cells of the part of the vascular strand derived from the pericycle, and secondary thickenings are visible on xylem walls (figs. 11, 12). Differentiation then proceeds from the root stele acropetally in the provascular strands. This differentiation is paralleled by differentiation of the nodule endodermis. About the time that differentiation of xylem and phloem begins in the provascular strand, the nodule endodermis starts to develop adjacent to the root endodermis. The peripheral cells at the base of the nodule develop wall thickenings, which are typical Casparian

bands. In the part of each vascular strand between the root endodermis and the nodule meristem, the bundle endodermis becomes differentiated as a layer continuous with the endodermal layer of the root (fig. 24). At the base of the vascular strand, the bundle endodermis and the xylem mature at about the same time, a little later than the nodule endodermis. Cross sections of the nodule show a slightly different order of differentiation near its apex. Here the first tissue to mature in the vascular strands is the bundle endodermis, which develops Casparian bands at about the level nearest the apex at which bacteroid tissue is distinguishable (figs. 27, 28). This differentiation is followed closely by maturation of the nodule endodermis, which develops typical Casparian strips first opposite the provascular strands (fig. 29), and then becomes continuous around the entire nodule. The phloem becomes recognizable by its paired cells with prominent nuclei, and thickening of the xylem walls begins at almost the same level, a few microns farther from the apex than the first differentiated nodule endodermis (fig. 30). The fully differentiated vascular strand is composed of scalariform xylem elements—all of which appear to be tracheids—that lie toward the outside of the nodule and, centripetal to the xylem, phloem consisting of sieve tubes, companion cells, and parenchyma. The conducting tissues are surrounded by a parenchymatous bundle sheath, and the whole bundle is surrounded by the vascular endodermis (figs. 29, 30). The inverse collateral arrangement of xylem and phloem in the bundle is established very near the nodule base (fig. 25). This is true even in a strand that is bicollateral at its base. In cases in which strands of phloem connect to the two phloem strands adjacent to a xylem arm



FIGS. 28-34.—Fig. 28, bundle at level just above that shown in fig. 27; *a*, Casparian bands in part of bundle endodermis. Fig. 29, bundle just below level in fig. 27; *a*, bundle endodermis; *b*, nodule endodermis; *c*, xylem with walls becoming thickened; *d*, phloem; *e*, bundle sheath. Fig. 30, longitudinal section of bundle from same nodule; *a*, bundle endodermis; *b*, xylem; *c*, sieve tube; *d*, companion cell; *e*, bundle sheath. Fig. 31, edge of nodule, showing $2n$ chromosome plate in nodule cortex. Fig. 32, portion of same, enlarged to show $2n$ ($= 14$) chromosome plate. Fig. 33, cell from nodule meristem of Canada pea showing 28 ($4n$) chromosomes. Fig. 34, part of provascular strand, showing one $2n$ and one $4n$ chromosome plate. Figs. 28-30, 32, 33, $\times 550$. Figs. 31, 34, $\times 250$.

in the root stele, they arise at a level slightly nearer the center of the nodule than the xylem. The orientation of the two strands changes through an angle of 90° so that one comes to lie above the other and both are nearer the axis of the nodule than the xylem. As they differentiate apically, these two strands join to form the single phloem strand found throughout most of the length of the vascular bundle. At the very base of the nodule there is some cambial activity in the bundle, and numerous tracheids are added by the cambium and by the maturation of pericyclic derivatives to make the connecting xylem mass conical in shape. The phloem of the nodular bundles does not reach the stage of maturity found in the root stele; the nuclei remain prominent in the sieve tubes, and slime bodies are not conspicuous.

The general arrangement of vascular tissues and the branching of the bundles of nodules were described by VUILLEMIN (26) for several species of leguminous plants. He did not observe the nodule endodermis but did picture the endodermal layer around each bundle. He considered the nodule vascular system a simple polystele with inverted bundles. DANGEARD (8) observed both the nodule endodermis and the bundle endodermis in a number of Leguminosae, including *Pisum*. In only one case, which he dismissed, did he observe levels of the nodule at which the continuity of the root endodermis across the nodule base was visible. For this reason, he interpreted the nodule endodermis as a stretched root endodermis and considered the nodule pericyclic in origin. THORNTON and his co-workers (4, 21, 23) noted the presence of the two types of endodermis in the nodules of alfalfa but did not trace their development. FRAZER (11) studied in detail the endodermal layers in mature

nodules of clover, broad bean, soybean, and pea, varieties "Maple" and "Gladstone"; but she concluded that the outer layer was not a typical endodermis because she found no walls with Casparian bands but only completely suberized walls. THORNTON applied the name "lateral endodermis" and FRAZER the name "common endodermis" to the outer layer that is called here the "nodule endodermis." FRAZER pointed out that the term "lateral" is hardly an accurate description of the outer endodermis in nodules of the spherical type. Because "common" is used to refer to vascular tissues in stems, the term "nodule endodermis" is used in the present study as more nearly descriptive of the tissue in question. The term "vascular endodermis" or "bundle endodermis" seems adequate for the endodermal layer surrounding the vascular strand. In the varieties of pea used in the present investigation, both the vascular and the outer endodermal layers of the nodule as well as the root endodermis show typical Casparian bands and should be considered true endodermal layers. Their physiological significance has not been determined adequately (11), and, because their arrangement is unlike that found in any other plant organ, further physiological studies should be of interest.

This account of the vascular system of the pea nodule differs at several points from that given for soybean by BIEBERDORF (2). He found that, in the formation of the provascular strands, the first radial divisions occur in the cortical parenchyma cells at the base of the nodule and proceed both apically and back toward the root. In contrast, radial divisions that form provascular strands in pea proceed acropetally. Another difference is that BIEBERDORF found nodular bundles to be not collateral but made up of

scalariform xylem vessels "surrounded by parenchyma cells that are of a phloem-like nature." He observed considerable cambial activity in the nodule bundles of soybean, which is in contrast to the usually slight cambial activity in pea. In the present study there was no evidence that vascular bundles in the pea unite at the nodule apex as BIEBERDORF found that they do in soybean.

The necessity of boron for vascular differentiation in the nodule (4) and the peculiar effects of sodium nitrate upon its endodermal layers (23) suggest that further physiological studies are necessary for an understanding of the complex interrelationship of rhizobia and leguminous host plants of which nodule development is one result.

The chromosome number of nodule cells is of interest. Present observations agree with those of others (20, 30, 31) in showing that many cells in the nodule meristem are tetraploid, having twenty-eight chromosomes (fig. 33). In one case, however, a plate showing fourteen chromosomes was found near the center of the meristematic region of a fairly mature nodule. The cells in the nodule cortex were observed to be diploid (figs. 31, 32), as was noted by WIPF (29). In one case, diploid and tetraploid chromosome plates were observed in the same provascular strand (fig. 34). This raises an interesting question as to the nature of the tissues making up the vascular bundles. Additional information as to actual chromosome numbers in various parts of the nodule would be of very great interest. The lack of division figures in the bacteroid and some other regions has made such information relatively unavailable. Because of the tremendous variation in size of resting nuclei within the nodule (figs. 18, 19, 20), there is some doubt that their size can be considered a reliable

criterion of chromosome number in these cells.

From their observations on the origin of nodules, WIPF and COOPER (31) suggested that in the leguminous root only regions containing disomatic cortical cells develop into nodules and that the lack of proliferation of cortical cells near other infection strands may be the result of the absence of tetraploid cells in the vicinity. If this is the case, it would be of interest to know why only those diploid cells occurring near tetraploid cells divide in response to the presence of rhizobia. Carbohydrate supply has been suggested as another limiting factor to the number of infection strands that actually stimulate nodule development. Observations indicate that the number of nodules per unit length of pea root is greater in plants growing under conditions very favorable for photosynthesis; whether the number of naturally occurring disomatic cells is also increased under such conditions is not known. In view of the variety of substances that may upset mitosis and result in the production of polyploidy, the possibility that some substance secreted by the rhizobia may induce polyploidy in the nodule cells is not entirely eliminated. If the infection strands can infect only cells that are already disomatic, then the disomaty may be a result of some other characteristic of the cell, such as cytoplasmic viscosity, that also allows release of the bacteria into the host-cell cytoplasm. All these questions suggest lines for further study of the interrelations of rhizobia and the cells of their hosts.

Summary

1. The anatomy of the root of *Pisum sativum* in the region of nodule development is described.
2. Both the protophloem and the metaploem in the roots of plants of the variety Perfection are characterized by

sieve tubes with companion cells and sieve plates. In the variety Canada, roots show conspicuous dumbbell-shaped slime bodies in the sieve tubes.

3. Branch roots originate by divisions of pericyclic cells opposite one of the xylem arms.

4. Some radial divisions occur in the endodermal cells opposite developing branch roots, but the endodermal sheath remains continuous for only a short time and is soon ruptured near the base of the elongating lateral root.

5. Root nodules originate by proliferation of the cortical parenchyma cells in the vicinity of the rhizobial infection strand, which enters the root through a root hair and penetrates through three to five layers of cortical parenchyma cells.

6. The number of cells in the nodule increases at first by divisions throughout a spherical mass of cells, then by the action of an apically localized meristematic region.

7. Branches of the infection strand penetrate many of the cells in the central region of the developing nodule, and rhizobia are released into the cytoplasm of the host cells.

8. Divisions occur in some infected cells, but no division stages were observed in cells having many rhizobia in the cytoplasm.

9. The nodule becomes differentiated into an infected or bacteroid region, adjacent to the apical meristematic region, and an uninfected cortical parenchyma.

10. The root epidermis is broken as the nodule enlarges, but the nodule does not emerge from the root cortex.

11. Two main vascular strands differentiate at the base of the nodule and branch dichotomously to give from six to ten vascular bundles near the apex of the nodule.

12. At the level of the root at which a

nodule vascular strand differentiates, the pericyclic cells adjacent to a xylem arm and the endodermal cells in the same radii of the root divide first tangentially, then radially. The provascular strands become differentiated acropetally by radial divisions of cells in the uninfected cortical region of the nodule.

13. Maturation of vascular tissues in the nodule begins in the cells derived from the root pericycle and proceeds acropetally.

14. There are two endodermal layers in the nodule. An endodermis with typical Casparian bands surrounds each vascular strand and is termed the "vascular endodermis" or "bundle endodermis." An endodermal layer designated here as the "nodule endodermis" differentiates in the outermost layer of the uninfected cells of the nodule cortex and forms a cylinder adjacent to the larger stretched cells of the surrounding root cortex. The cells of this endodermal layer also have typical Casparian bands. Both endodermal layers join the endodermis of the root.

15. The number of chromosomes found in the cells of the nodule usually is twenty-eight ($4n$) in its meristem and fourteen ($2n$) in its cortical region. One cell near the center of the nodule meristem had fourteen chromosomes. Two cells in the same provascular strand had different numbers of chromosomes, one fourteen, one twenty-eight.

I wish to express my sincere appreciation for the helpful suggestions and criticism given in the course of this investigation by Professors EMMA L. FISK and ELIZABETH MCCOY of the University of Wisconsin. The photomicrographs were made by Mr. EUGENE HERRLING.

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RESPONSES OF PEA ROOTS TO APPLICATION OF CERTAIN GROWTH-REGULATING SUBSTANCES

LORA BOND¹

Introduction

The stimulus to cell proliferation in leguminous roots that ultimately results in nodule development has been the subject of considerable speculation. The root nodule is obviously related to bacterial infection (11), and for a time after the discovery of bacteria in the nodule no other stimulus than their presence was sought. In 1936 THIMANN (25) suggested a theory of nodule formation based upon a synthesis of several facts established by earlier experiments. According to his theory, the nodule is essentially a branch root stimulated to develop but prevented from elongating by some diffusible substance, probably indoleacetic acid, produced by the rhizobia. The direct experimental evidence that THIMANN gave in support of his theory is that (a) young nodules give a positive *Avena* test and (b) external application of indole-3-acetic acid induces swelling of pea roots and lateral root initiation. Other investigators have demonstrated that rhizobia produce indoleacetic acid if grown in a medium containing tryptophane (9, 12, 18); that nodules have a greater auxin content than the roots on which they are borne (19); and that rhizobial culture medium (22) or pure indoleacetic acid (20) will cause pericyclic proliferation and cortical hypertrophy. Indoleacetic acid also induces excessive branch root initiation in leguminous roots. Such evidence supports THIMANN's theory, but

KRAUS (14) pointed out that anatomical evidence also should be taken into account.

The nodule anatomy of several leguminous plants (alfalfa, bean, bur clover, pea, peanut, and soybean) has been studied in detail (26; 21; 23; 6, 10, 27; 1; 4). Except in peanut, the root tissue that proliferates and contributes to the nodule structure is the cortical parenchyma. Secondary roots in leguminous plants that have been examined (6, 7) are pericyclic in origin. The difference in origin of these two structures indicates that they are not homologous. Even in peanut, in which the nodule does arise in the pericycle, there are anatomical differences between nodules and lateral roots (1). The idea that the tubercle or nodule is a potential lateral root, the development of which has been arrested, is not supported by literature dealing with nodule morphology. The question remains whether some specific substance produced by the rhizobia stimulates the proliferation of the root cortex.

REVIEW OF LITERATURE

There are few reports on the effects of application of growth-regulating compounds to roots; certainly there have been few such extensive and careful histological studies as those made by KRAUS and his coworkers (13, 14, 15, 16) on decapitated stems and other aerial parts of bean plants.

The cessation of elongation and the increase in diameter of roots of plants grown from seeds or seedlings treated with colchicine, chloral hydrate, or

¹ Investigation carried on at the University of Wisconsin, where the author was a Wisconsin Alumni Research Foundation Research Assistant in the Department of Botany.

growth substances has been noted by many workers, cited by BOND (5) and by CARLTON (8).

ZIMMERMAN and HITCHCOCK (28) found that when aerial roots of a tropical grape (*Cissus sicyoides* L. var. *jaquini* Planch.) were treated with various "root-forming substances" (alpha-naphthaleneacetic acid, indoleacetic acid, indolepropionic acid, indolebutyric acid, delta-[3-indolyl]-valeric acid, and phenylacetic acid) in aqueous solution or in lanolin preparation, responses were bending, retardation in extension, increase in diameter, and, within 3 days, the initiation of branch roots. They found application of lanolin mixtures farther away from the tip than the region of elongation to be ineffective unless the root was scraped first, in which case lateral roots were initiated just behind the treated area. PFEIFFER (24) observed sections of roots of the same tropical grape which had been treated with indolebutyric acid or other compounds. The branch roots that developed on the aerial roots following chemical treatment were typical lateral roots arising in the pericycle; the tissue giving rise to each young root lay entirely within the endodermis of the main root. ZIMMERMAN and HITCHCOCK (29), in further experiments on the responses of aerial roots of *Cissus*, found that both phenoxy and naphthoxy compounds induced unusual swellings that developed into fasciated rows of branch roots opposite the vascular strands. The vascular structure was maintained in each lateral root primordium of the fasciated row of branch roots, and the vascular tissue showed that each fasciated wing was composed of six or more roots.

CARLTON (8) made detailed anatomical studies of treated roots of several bulb-forming monocotyledonous plants (*Allium cepa*, *Narcissus* var. Paper White,

Tulipa vars. John Ruskin and Louis XIV). The bulbs were allowed to develop roots in a three-salt nutrient solution; individual bulbs were then transferred for 24-72 hours to a nutrient solution containing one of six growth-regulating substances (alpha-naphthaleneacetic acid, indole-3-acetic acid, indole-3-butyric acid, beta-naphthoxyacetic acid, alpha-naphthyl acetamide, and tryptophane). Roots were sectioned at different intervals after being transferred back to the ordinary nutrient solution. CARLTON observed that root tumors which developed differed with both the kind of plant and the chemical used. In *Narcissus* only slight proliferation was observed and that was in endodermal cells. In *Allium* tumors resulted chiefly from hypertrophy of cortical cells and from proliferation of pericycle cells, with or without formation of definite, organized branch-root primordia.

There have been a few investigations of root responses of leguminous plants to growth-regulating chemicals. These include the studies of THIMANN (25) on pea and LINK *et al.* (20) on bean. The roots on their plants curved and developed irregular enlargements in response to surface application of indoleacetic acid in lanolin; no detailed histological studies were made in either of these cases. BEAL (2, 3) found that certain chlorophenoxy compounds, when applied to leaves or stems of sweet pea or bean, induced marked proliferation of certain tissues in their roots.

Material and methods

Two varieties of cultivated pea (*Pisum sativum* L.) were used: Canada field pea and Perfection garden pea. Because the study was undertaken in order to compare the effects of growth-regulating substances with the effects of rhizobia in

stimulating nodule production, the plants were grown under bacteriologically controlled conditions to insure the absence of rhizobia from the cultures. Seeds were sterilized by a method previously described (6) and were allowed to soak overnight in sterile distilled water. Germinating seeds were then placed in culture jars (fig. 1) that had been sterilized by autoclaving. These jars were cylindrical, $3\frac{1}{4}$ inches in diameter and 8 inches high. Each was filled to a depth of 1 inch with a Crone's salts nutrient solution with $\frac{1}{2}\%$ agar added to give a semisolid medium. Half a Petri dish was used as the cover for each jar, and a band of plugging cotton was inserted between the jar rim and the lid to allow some interchange of gases. Twelve cultures were set up, six of Canada peas and six of Perfection peas. The seeds, usually five per jar, were placed carefully on the surface of the medium, with the hypocotyl pointing down. The jars were kept in a dark room for 2 days, until the primary root had penetrated into the culture medium and the plumule had emerged from the seed coat. Then a black paper jacket 5 inches wide was fastened around the lower part of each jar, and the cultures were transferred to the greenhouse.

A week later the roots were treated. Five growth-regulating substances were used: indoleacetic acid, asparagine, tryptophane, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,3,5-triiodobenzoic acid, each at a concentration of 1% in lanolin, except asparagine, used at 3%. In most cases a small block of agar containing a primary or secondary root tip was cut out aseptically, and the cut tip was measured to determine how far from the tip the surface to be treated lay. The tip was discarded. Then a pellet of lanolin containing the chemical was placed on the cut surface of the attached root ex-

posed by removal of the agar block. Lanolin mixture was applied to the tips of a few uncut roots. Some roots were cut and not treated further, some were cut and treated with plain lanolin, and plain lanolin was applied to the tips of some uncut roots. Each substance was applied to the roots of Canada peas in one jar and

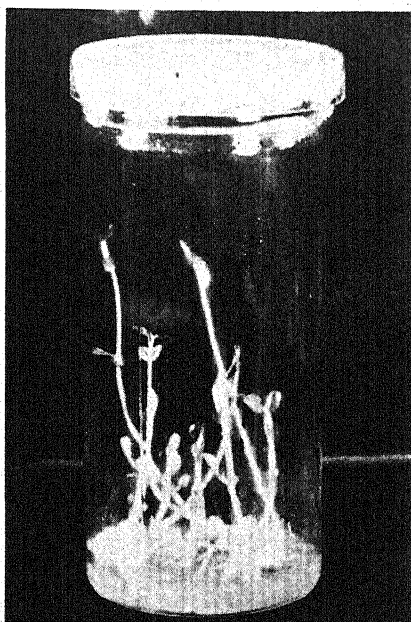


FIG. 1.—Culture jar used for growing plants treated with growth substances under bacteriologically controlled conditions.

to Perfection peas in another. In addition, one container of plants of each variety was left untreated, except for three root tips of one plant of each that were inserted into vials of liquid filtrate from a culture of rhizobia.

Treated roots, any other abnormal-looking roots from the same plants, and controls were collected 1 week after application of the chemicals and fixed in Karpechenko's modification of Nava-shin's solution, dehydrated, cleared and infiltrated by the cedar-oil method, and imbedded in paraffin. Sections cut 10 μ

thick were stained with safranin and Delafield's haematoxylin. A few roots were cleared in *eau de Javelle*, stained in basic fuchsin, dehydrated, and mounted whole.

Results and discussion

2,4,5-TRICHLOROPHENOXYACETIC ACID.

—This acid induced more striking re-

only in the distal portions, so that there was a greater difference in diameter between the root tip and other parts of the organ than in the cut roots (fig. 2). On the same plants there was a marked effect upon short secondary roots, which appeared stubby and of a characteristic mucronate shape (fig. 2). External observations indicated that the roots vir-

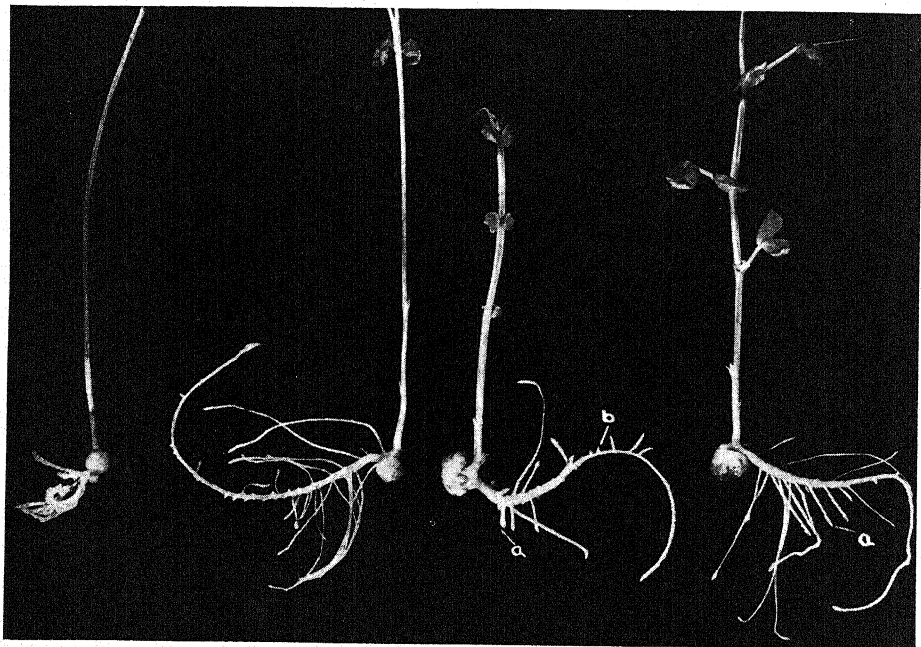


FIG. 2.—Plants 1 week after application of 2,4,5-T; variety Canada at left, Perfection at right; a, cut roots; b, short "mucronate" secondary root. $\times \frac{1}{2}$.

sponses than the other four growth substances used in these experiments. The cut secondary roots directly treated with this substance became thickened throughout their length, but the greatest increase in diameter occurred within 2 or 3 mm. from the treated surface. These roots were enlarged sufficiently to appear abnormal macroscopically (fig. 2). Even more apparent were the enlargements at the uncult tips of the primary and other fairly long secondary roots of the same plants. These uncut roots were thickened

actually stopped elongating following application of the substance.

There were apparently two factors involved in the spread of this growth substance from the point of application to other parts of the plant and to other plants. One factor was transport within the plant. Evidence that this may occur is found in the experiments of BEAL (2), who observed "telemorphic effects" induced in the root system of the sweet pea following application of a related compound, 4-chlorophenoxyacetic acid, to

the leaflets or stems. The second factor was diffusion of the substance through the semisolid agar medium in which the plants were grown. 2,4,5-T evidently dissolved out into the medium from the lanolin mixture applied to the roots. In the culture of Canada peas so treated, the lanolin mixture was not applied to one plant (extreme left in fig. 2) because it had too few branch roots when the other plants were treated. Its roots, however, later showed abnormalities similar to those of the other plants. The changes were more pronounced because they occurred at a different stage in root development.

Mounts of whole roots affected by 2,4,5-T showed that increase in root diameter resulted chiefly from irregular enlargement of the stelar region. The variations in diameter increased with distance from the tip, and enlarged portions were associated with secondary-root primordia at higher levels.

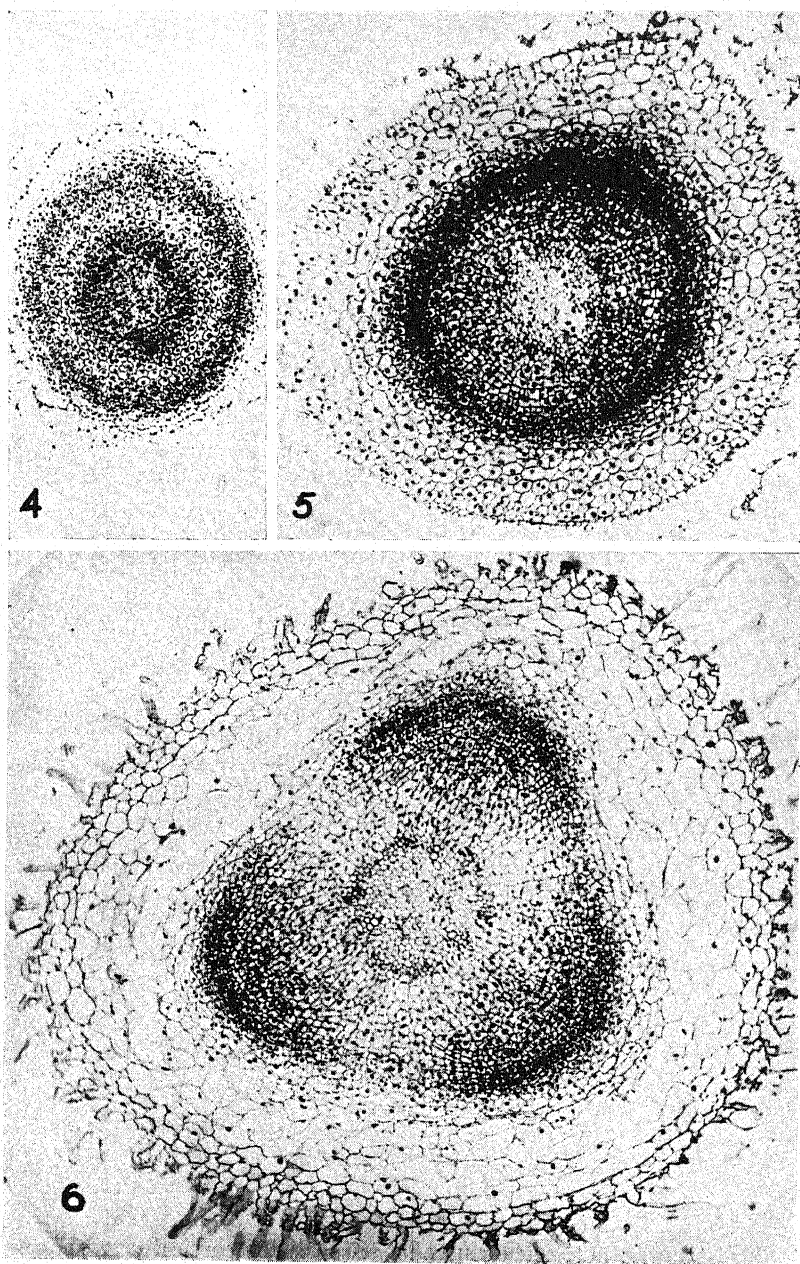
Sections of abnormal pea roots revealed that tissue proliferation induced by 2,4,5-T was somewhat similar to that induced by 4-chlorophenoxyacetic acid in sweet pea (2). Both longitudinal and transverse sections showed that root tissues obviously affected by 2,4,5-T differed according to the stages of maturity reached at the time of application. In longitudinal section, the root cap cells appeared more nearly round and were arranged in more regular rows than is usual in pea roots (fig. 3). Active division of cortical cells was found at a greater distance from the tip in affected roots than is usual in normal roots (figs. 3, 4). The greatest meristematic activity occurred in the pericyclic region. From 0.5 mm. to 0.75 mm. from the root tip, the pericycle appeared in cross section as a ring of very small cells, with dense protoplasm, encircling the immature vascular

tissues and surrounded by a cortex of larger cells that were still dividing (fig. 4). At a higher level, 1 mm. from the tip, the band made up of radial rows of pericyclic cells was much wider, accounting



FIG. 3.—Longitudinal section of root of Perfection pea treated with 2,4,5-T; a, hypertrophied cells. $\times 70$.

for almost all the increase in root diameter (fig. 5). At this level, protoxylem was differentiated, and there was little evidence of proliferation in the vascular tissues. The cortical cells showed only a few divisions. No distinct endodermal layer with Casparian bands was visible, and it appeared that some proliferation of the endodermis may have been associated with the marked meristematic ac-



FIGS. 4-6.—Transverse sections at successive levels from near tip to most enlarged region of root from same plant as in fig. 3. $\times 100$.

tivity of the pericycle. At a little higher level, 1.5 mm. from the tip, there was still no evidence of proliferation in the vascular tissue, but the band of pericyclic cells was even wider, and the most active divisions were localized opposite one or more of the xylem arms (figs. 3, 6, 7). At this level, approximately that of maximum diameter in the enlarged portion of the root, there was no meristematic activity in the cortex, and many of the cortical cells were stretched or torn by the centrifugal growth of masses of pericyclic cells. The number of root hairs on the part of the root 1 to 2 mm. from the tip, normally the region of maturation, was quite large, with the greatest number at the level of maximum diameter. Marked hypertrophy of epidermal cells was also evident (fig. 3). In some parts of the root, the outermost cells were at the ends of radial rows of two to four cells that appeared to have arisen earlier by tangential divisions of epidermal cells. In the mature region, there was less tissue proliferation in response to 2,4,5-T, and it was limited to the vicinity of young branch roots. Meristematic activity in the cortical parenchyma of the base of partly emerged secondary roots and in the near-by pericyclic cells of the main root was responsible for the development of the "collar" that made the root appear mucronate in shape (fig. 8).

The response of the root tissues at different levels indicates that the pericyclic cells retain the power of division longer than do the cells of other tissues.

The vascular tissues did not mature beyond the stage they had reached when 2,4,5-T was applied. This point is of interest in connection with the application of phenoxy compounds as herbicides.

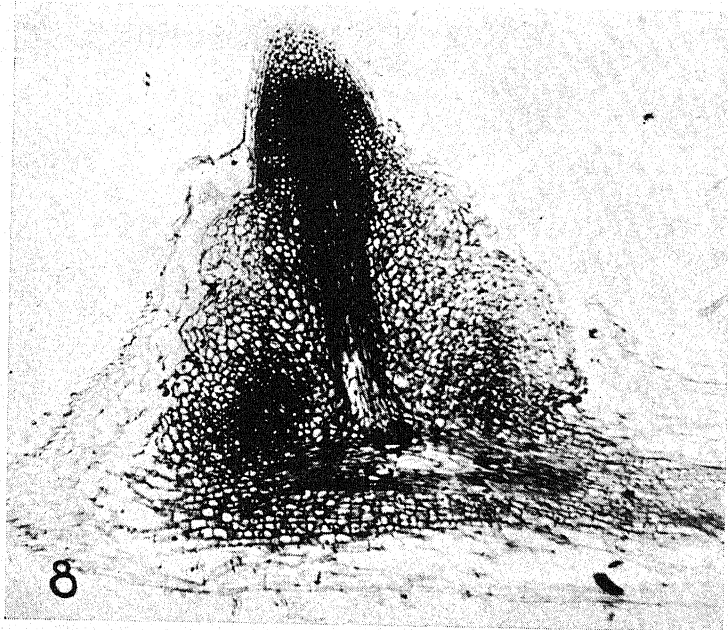
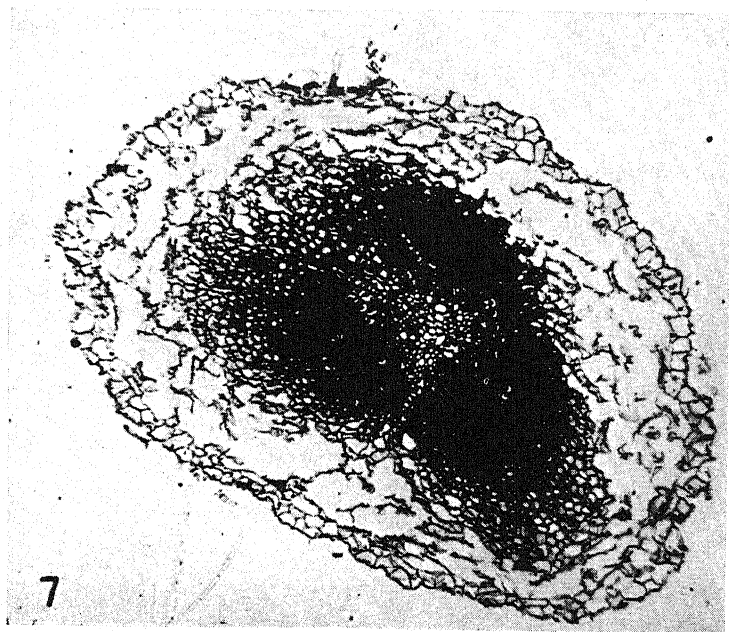
TRYPTOPHANE.—In material treated with this substance, the roots were not so obviously abnormal in external appear-

ance as were those treated with 2,4,5-T. The tips of treated cut roots were slightly thickened, and uncut treated roots were visibly enlarged, but untreated roots on the same plants were not affected.

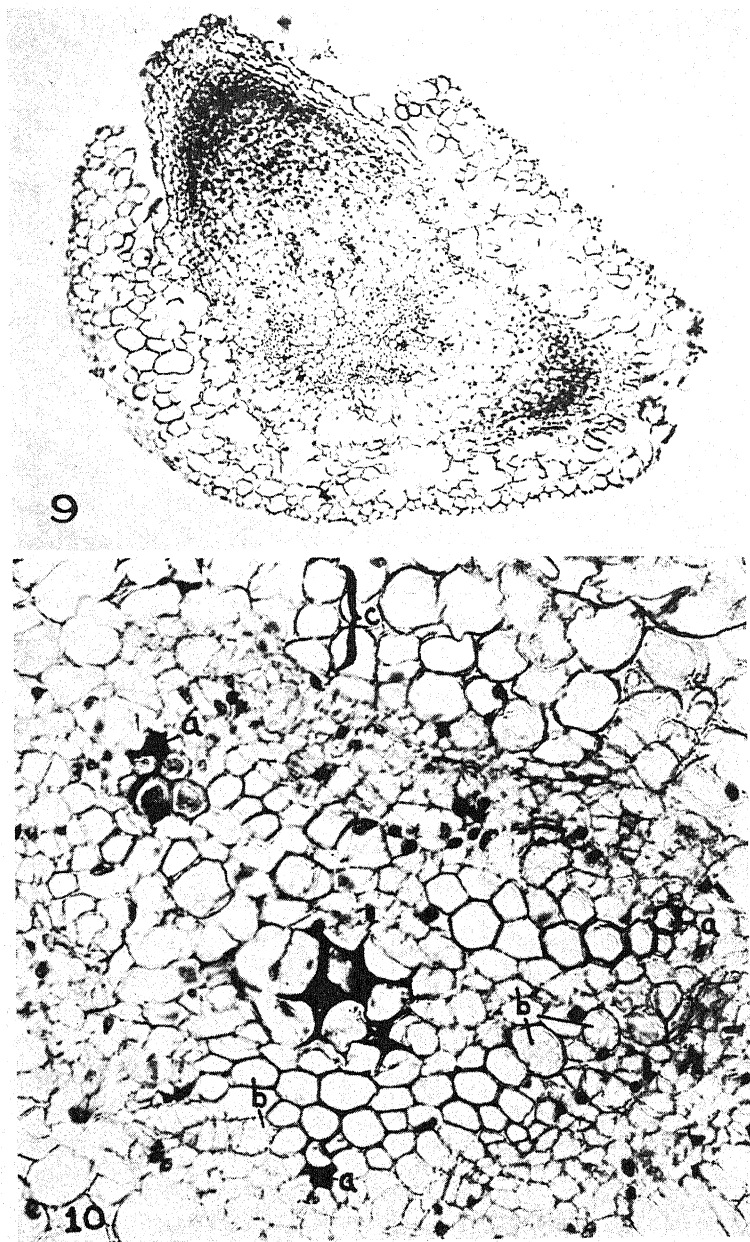
Sections of tryptophane-treated roots showed several very marked differences in development from roots treated with the phenoxy compound. As in the latter, there was an increase in diameter of the stele, with consequent tearing of cortical cells (fig. 9). There were fewer divisions of pericyclic cells that resulted in definite radial rows of two to four cells, and one or more divisions occurred in endodermal cells (figs. 10, 12). The increase in stelar tissue was brought about in tryptophane-treated material chiefly by proliferation of xylem parenchyma, accompanied by some cambial activity. The phloem proliferated very little, if at all, and it became somewhat crushed by the proliferating xylem. Numerous cells differentiated as scalariform tracheids in the stele, especially those derived from the xylem parenchyma. The protoxylem elements became plugged (fig. 10). Tissue maturation proceeded nearer to the tip than in untreated roots (fig. 11).

The response to tryptophane was much more marked in pea roots than in roots of *Allium*, *Tulipa*, and *Narcissus* observed by CARLTON (8). In *Allium* there was little change in treated roots except that the endodermal cells divided tangentially. In *Tulipa* some cells of the proliferated endodermis "take on the appearance of wound tracheids." In roots of *Narcissus* there were a few divisions of pericyclic cells as well as of endodermal cells.

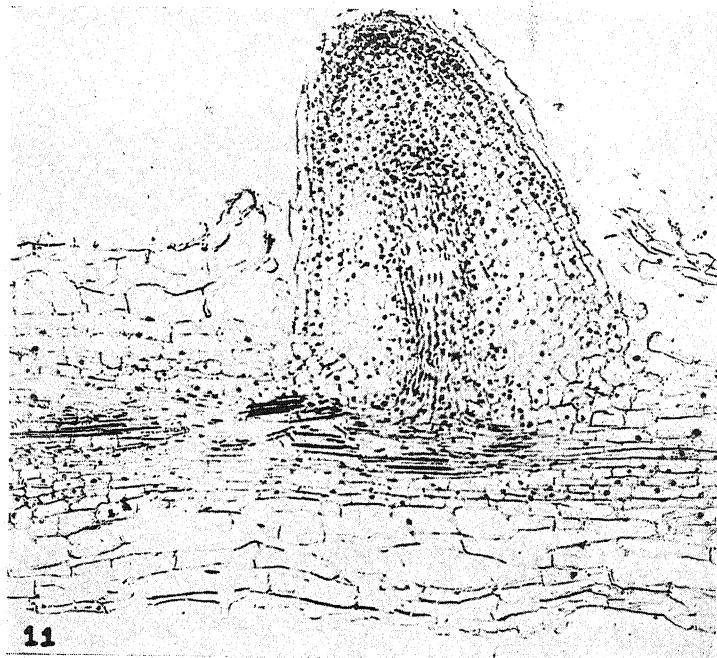
The response of pea roots to tryptophane was in some respects similar to, and in other respects quite different from, the response to tryptophane observed in bean stems by KRAUS (14). In



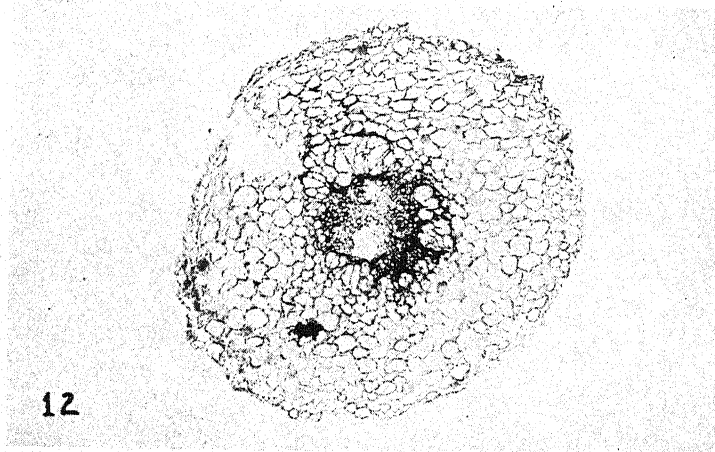
FIGS. 7-8.—Fig. 7, transverse section of root of Canada pea treated with 2,4,5-T. $\times 100$. Fig. 8, longitudinal section of root of Perfection pea treated with 2,4,5-T, showing "mucronate" lateral root. $\times 100$.



FIGS. 9-10.—Fig. 9, transverse section through enlarged portion of root of Canada pea treated with tryptophane. $\times 70$. Fig. 10, stellar region of root shown at lower magnification in fig. 9; *a*, protoxylem, partly plugged; *b*, scalariform tracheids; *c*, row of pericyclic derivatives. $\times 450$.



11



12

FIGS. 11-12.—Fig. 11, longitudinal section of root of Canada pea treated with tryptophane, showing abnormal maturation in lateral root. $\times 70$. Fig. 12, transverse section of root of Perfection pea treated with tryptophane. $\times 100$.

bean stems, as in pea roots, the cambium underwent many divisions. There was enlargement and some division of epidermal cells in the bean stems, and the cortical parenchyma cells divided to some extent. These responses were accompanied by an extensive proliferation of ray cells. The most striking effect in bean stems, the very active proliferation of endodermal cells, was less prominent in pea roots.

Three responses of pea roots to tryptophane are of interest because of similarities to nodule proliferation. First, the cortical cells remained in a continuous layer over root primordia much longer than is normal; second, there was vascular differentiation in the proliferated cells; and third, some division of both endodermal and pericyclic cells occurred. There was one great difference between the response to tryptophane and the effects of rhizobia in root tissues. In the tryptophane-treated material there was almost no proliferation of cortical cells, which form most of the nodule mass. Another difference was that vascular differentiation did not follow a definite pattern, as it does in the nodule, but was quite irregular.

OTHER SUBSTANCES.—Roots treated with indoleacetic acid increased slightly in diameter as a result of hypertrophy of cortical cells in the region of elongation. There was also some initiation of lateral roots by pericyclic activity. In roots treated with the filtrate from a culture of rhizobia, there was some stimulation of branch-root formation.

Roots treated with asparagine or with 2,3,5-triiodobenzoic acid showed almost no changes. Part of the reason for this lack of response may be that all the roots directly treated with those substances had the tips cut off, whereas in both the 2,4,5-T- and the tryptophane-treated

material greater changes appeared in the uncut treated roots included in those series than in cut roots. Changes in the treated cut roots were far less marked than the responses to tryptophane reported for decapitated bean stems by KRAUS (14). The only cut roots that showed very great reactions were those treated with 2,4,5-T.

Both lots of treated pea roots described in detail above—those treated with 2,4,5-T and those treated with tryptophane—differed in two respects from the roots of *Cissus* (24) and *Allium* (17) treated with indoleacetic, indolepropionic, indolebutyric, or alpha-naphthaleneacetic acid. First, the responses were not primarily the initiation of branch roots as in *Cissus* and *Allium*, and, second, increase in the diameter of pea roots treated with tryptophane or with 2,4,5-T was brought about chiefly by an increase in cell number rather than by cell enlargement. CARLTON (8) observed almost no increase in cell number, except near the meristematic region and in branch primordia, in roots of *Allium*, *Narcissus*, and *Tulipa* enlarged as a result of treatment with indole compounds.

It may be concluded from these results that none of the growth substances used in these experiments stimulates the type of tissue proliferation found in the formation of root nodules. Notably, proliferation of cortical cells was almost entirely lacking in all cases. This does not eliminate the possibility that the effect of rhizobia is brought about by some diffusible substance, not yet identified, that is produced by the bacteria.

Summary

1. Pea roots with their tips cut off showed marked response to application of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in lanolin and a less marked

one to tryptophane. Only slight reactions followed similar application of asparagine, indoleacetic acid, 2,3,5-triiodobenzoic acid, or filtrate from a culture of rhizobia.

2. Uncut roots enlarged more than cut roots following direct application of tryptophane or 2,4,5-T to each. Uncut roots enlarged more than cut roots on the same plant, following application of 2,4,5-T to the latter.

3. Roots of pea showed the following effects after treatment with 2,4,5-T: (a) an increase in diameter brought about largely by meristematic activity of the pericycle, accompanied by some proliferation of the endodermis; (b) active division of cortical parenchyma near the meristematic region; (c) in the enlarged region, stretched and torn cortical parenchyma cells; (d) in the mature region, meristematic activity of the pericycle limited to the vicinity of branch roots; (e) hypertrophy of epidermal cells; (f) an increase in number of root hairs, especially at the level of greatest diameter of the enlarged root; (g) failure in differentiation of vascular tissues beyond the stage reached at the time of application of the growth substance.

4. Pea roots showed the following effects from application of tryptophane: (a) an increase in diameter brought about chiefly by proliferation of xylem parenchyma and cambium; (b) some divisions in pericycle and endodermis; (c) scattered cells in the stelar region maturing as scalariform tracheids.

5. Tryptophane-treated roots resembled those with developing nodules in that there was some division of both pericyclic and endodermal cells and in that there was differentiation of scalariform tracheids in the proliferated tissues of the stele.

6. In pea roots none of the growth substances used stimulated division of the cortical parenchyma cells, in which most of the proliferation takes place in developing nodules.

I wish to express my sincere appreciation for the helpful suggestions and criticism given in the course of this investigation by Professors EMMA L. FISK and ELIZABETH MCCOY of the University of Wisconsin. The photomicrographs were made by Mr. EUGENE HERRLING.

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ABSCISSION AND OTHER RESPONSES INDUCED BY 2,3,5-TRIIODOBENZOIC ACID IN BEAN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 598

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Introduction

2,3,5-Triiodobenzoic acid has been classified (13) as a plant growth-regulating substance which is active in inducing modification of organs but is inactive in causing cell elongation or root formation. In initial tests of this substance for physiological activity, ZIMMERMAN and HITCHCOCK (14, 15) observed marked disturbance in the correlation of organs in tomato plants treated with the acid.

They reported that vegetative growth and flowering habits were modified.

In trials of various substances for herbicidal purposes, KRAUS and MITCHELL (6) found that 2,3,5-triiodobenzoic acid induced changes mainly in the vegetative character of treated bean plants. In the stem, internodal elongation was checked, and growth of terminal and axillary buds was affected. Leaves from these buds were dwarfed and curled, and

the surface was sometimes pebbled. The treatment failed to induce tumor formation, nor was the development of roots reported. In regard to flowering, they reported that the numbers of flowers in treated bean plants were not appreciably fewer than those of controls. The total growth was increased and the flowering period prolonged.

TUMANOV and LIZANDR (10) found distinct variation in the sensitivity to the acid of the six different kinds of plants with which they experimented, *Medicago sativa* being the most sensitive. Moreover, the response to treatment varied with the photoperiod. Vegetative plants of alfalfa grown on short day showed modification of organs in the growing points. Alfalfa grown under long photoperiod and treated just before flowering gave an increased seed yield. Experiments by GALSTON (3), designed to determine the effect of this substance on flowering in soybean, also indicated different responses in growth according to the photoperiod. Soybeans photoperiodically maintained in a vegetative condition showed modification of organs and abscission of buds but no induction of flowers following treatment with the compound. Plants in which flowering had been photoperiodically induced, however, showed a significantly increased number of flower buds after treatment with this substance. GALSTON concluded that 2,3,5-triiodobenzoic acid possesses anti-auxinic activity of some sort but does not possess florigenic properties. He discussed a possible functional association of hormones in which auxin promotes general vegetative growth and counteracts florigen which favors flowering. On this basis lowered auxin levels following treatment with the anti-auxinic 2,3,5-triiodobenzoic acid would favor flowering of photoinduced soybeans but would not

induce flowering in the vegetative condition.

It is evident that the character of response resulting from treatment with 2,3,5-triiodobenzoic acid differs from those to other growth-regulating substances already reported in the series (5, 11) of experiments on the bean plant, *Phaseolus vulgaris* var. Red Kidney. The lack of cell elongation and the absence of adventitious roots and tumor formation as responses to 2,3,5-triiodobenzoic acid separate it from such substances as indoleacetic and naphthaleneacetic acids and the substituted phenoxy compounds, although both 2,3,5-triiodobenzoic acid and the phenoxy compounds induce formative or teleomorphic activity. The present paper reports (1) a series of general observations on the growth and flowering habits of the bean as modified by treatment with 2,3,5-triiodobenzoic acid and (2) the results of a histological study made to determine the tissues and areas affected by the acid and the nature of the anatomical changes which underlie the larger morphological modifications.

Methods

DECAPITATION AND RINGING.—Plants of Red Kidney bean were grown in the greenhouse in the spring of 1947. Young plants, selected for uniformity, were treated when the first trifoliate leaf was expanding and the second trifoliate was unfolding from the bud. 2,3,5-Triiodobenzoic acid mixed in a 2% concentration in lanolin was applied in two different ways. In one lot the plants were decapitated by severing the second internode in the same manner used in earlier experiments with the bean (5). The lanolin paste containing the growth-regulator was applied immediately over the cut surface. Similarly, decapitated control plants received an application of lanolin

alone. The remaining plants were not decapitated. Instead, the 2,3,5-triiodobenzoic acid-lanolin mixture was applied with a glass rod in a narrow band ringing the middle of the second internode. Control plants received a similar application of lanolin only. Observations and collections of material for histological examination were made over a period of 30 days. Material fixed in Navashin's fluid was imbedded by the butyl alcohol-paraffin method. Sections cut at 12 or 15 μ were stained with a modified triple stain.

SPRAYING.—In addition to the experiments in the greenhouse, plants were grown in the garden during the summer of 1947. They were sprayed with 1.0, 0.5, and 0.1% concentrations of the ammonium salt of 2,3,5-triiodobenzoic acid in water. The ammonium salt is more soluble in water than the acid. It was prepared by allowing ammonium hydroxide to react with the acid in proper quantities to make up the 1% concentration in water. The other two concentrations were prepared by dilution. All three solutions were used as aqueous sprays. In addition, three emulsion sprays were used. The base mixture of the commercial "Weed-No-More" was added in 1% concentration to the respective concentrations of the aqueous solutions. This base mixture contained no growth-regulator and was used as an emulsifier and a wetting agent. The selected spray was applied over the whole plant with a DeVilbiss atomizer. Wetness without running or accumulation of the spray in drops was considered desirable coverage.

One half of the planting received the aqueous sprays, the other half the emulsion sprays. In each half, four plots were established. Three plots received the three different concentrations (1.0, 0.5,

0.1%) of the ammonium salt, respectively, and the fourth, as a control plot, received either a spray of water or an emulsion spray of water containing 1% of the base mixture. Each plot consisted of two rows of plants. One row in each plot was sprayed 13 days after planting when the plants were in a stage similar to that of the plants treated in the greenhouse. This treatment will be referred to as the "young stage." Plants of the second row were treated 27 days after planting when flower buds were becoming evident. This will be known as the "old stage." All plants were observed for gross effects on the vegetative and flowering habits. Collections of material for various measurements were made over a period of 3 months. During this period of observation several weeks of unusually dry and hot weather presented rather unfavorable growing conditions, although this was not serious enough to cause actual injury in the control plants.

Results

GROSS EFFECTS

In the present experiments response to 2,3,5-triiodobenzoic acid by decapitated plants was relatively limited. Similar localization of response by decapitated plants has been observed with other growth-regulating substances. Responses to ringing and spraying with 2,3,5-triiodobenzoic acid were teleomorphic and occurred over large portions of the treated plant.¹ The teleomorphic effects were similar in both treatments and appeared to be distinctive for this substance.

DECAPITATION.—Discoloration was the earliest evidence of change in the decapitated stem tips. During the first 3

¹ In this report the term "treated" refers to treatment with 2,3,5-triiodobenzoic acid irrespective of the method of application.

days after application the cut surface of control stem tips paled or became slightly brown. Further development was limited, and enlargement occurred in only a few stems. In plants treated with the growth-regulator the cut surface darkened to a characteristic rusty-red color. Below the treated surface and downward for 0.5 mm. or more the stem color changed to a light green. By the fourth or fifth day slight enlargement became evident in some of the treated stems and continued through the eighth and ninth days. At this time most of these stem tips showed some degree of tumor formation. The tumors were shallow and generally small, although they varied considerably in size. Enlargement was greatest just below the cut surface and downward along the ridges of the vascular bundles. Frequently these ridges also showed the characteristic rusty-red color. The tumors continued to grow slowly. By the thirtieth day they appeared to reach a relatively inactive condition but were still firm and green. In none of the tumors were there protuberances indicative of root formation. Throughout the experiment the tumors were insignificant in size and in no way comparable in appearance with the tumors induced by indoleacetic or 2,4-dichlorophenoxyacetic acids (5, 8).

In addition to response at the decapitated tips, slight and delayed effects of treatment were observed in the development of buds in the axils of the simple leaves. In both treated and control plants, growth was redirected into these buds following decapitation. By the ninth day the first trifoliate leaf on the axillary shoot was considerably expanded and the young leaflets of the second trifoliate leaf were lengthening. In some treated plants these young leaflets showed epinasty with the tips of the

blades markedly curled downward. This response to treatment was of short duration, and further growth was similar in control and treated plants.

RINGING.—In contrast to the local effects induced by 2,3,5-triiodobenzoic acid in decapitated plants, application of the growth-regulator by ringing the second internode resulted mainly in teleomorphic effects. Response at the point of application consisted of streaking or burning in that portion of the internode covered by the lanolin containing the growth-regulator. The streaks occurred principally along the ridges over the vascular bundles. The discoloration was the rusty red already noted as a characteristic response to 2,3,5-triiodobenzoic acid treatment. These streaks appeared by the third or fourth day and were present to the end of the period of observation. No swelling or tumor formation was apparent. In a few plants a slight bending of the stem occurred at the level of ringing.

Teleomorphic effects occurred first in the leaves of the treated plants and were evident by the third day after ringing. The degree of response appeared to be closely related to the maturity of the leaf at the time of application. Fully expanded leaves (the heart-shaped, simple leaves and the first trifoliate leaf) showed little or no response. Partially expanded leaves (the second trifoliate in many of the plants) showed downward curvature of the tips of the leaflets. The lateral margins of the blades rolled inward, and frequently the surface became pebbled. Slightly expanded leaves, scarcely more than 1 cm. long at the time of ringing (in some plants the third trifoliate leaf and in others the second), displayed marked epinasty of the blade tips by 3 days. At the same time a silvered hairiness readily distinguished these leaves from those of

similar size in the control plants. Very young, unexpanded leaves (folded in the bud at the time of ringing) began to show epinasty and hairiness by the seventh day and were considerably dwarfed as compared with the controls.

The fate of the leaves varied with the intensity of response. Those only slightly affected appeared to outgrow the epinastic and retarding effects of the acid. Leaves which were somewhat more affected remained green but were dwarfed in size and continued to show epinasty. The more seriously modified leaves, such as the young ones near the bud, did not recover. By the sixth day they were a dull green in color and appeared to be wilted. On the seventh day some of the leaflet blades (see fig. 3*B*), or even the whole leaf, fell from the plant at the slightest touch. Abscission of other affected leaflets occurred the following days (cf. fig. 3*C*).

That 2,3,5-triiodobenzoic acid has a distinctive effect on young tissues is again demonstrated by the severe response exhibited in the buds. This effect was first noticeable in the terminal bud 3 or 4 days after ringing. Growth of the stem just below the bud was checked both in the elongation of internodes and in the increase in stem diameter. By the sixth day definite injury was indicated by discoloration of the terminal growth. There was a sharp demarcation of this color change in one of the partially elongated internodes a short distance below the bud. The color intensified and darkened to a brownish green during the next several days. At the same time the young leaves attached to this portion of the stem were collapsing and even abscising. On the ninth day or shortly thereafter the whole terminal bud abscised, often carrying with it the third and fourth trifoliate leaves. Among the

plants observed in detail in the greenhouse, this abscission of the main axis occurred most frequently in the fourth internode (see fig. 9*B*) but was also common in the third and fifth internodes. In a few plants less severely affected, terminal buds recovered, although later growth was dwarfed.

Stimulation of axillary growth was associated with the loss of apical dominance in treated plants. Under ordinary conditions of growth, however, the bushbean also shows considerable development of axillary structures, especially from the upper nodes—the fourth, fifth, and sixth. The buds at these nodes frequently develop as strong vegetative shoots which may form a major portion of the top growth in older plants. In addition to the axillary bud in any given axil, one or two accessory buds may develop (fig. 10*A*). These are commonly floral buds. Ordinarily the greatest growth of the lateral shoots follows termination of growth in the main axis by formation of a flower cluster, a characteristic occurrence above the seventh node. This pattern of termination in a flower cluster also occurs in the axillary shoots.

Treatment with 2,3,5-triiodobenzoic acid greatly modified this normal pattern of growth. By the fifth day development of axillary structures definitely exceeded that of control plants. At this time the loss of apical dominance in the treated plants was evidenced by the stem discoloration below the bud. In treated plants axillary shoots developed at the second, third, and fourth nodes. The shoots in the axils of the pair of simple leaves at the second node were frequently the most vigorous and later formed the major portion of the plant. The second node was below the level of ringing. In comparable control plants axillary buds

were present but undeveloped at these lower nodes.

The teleomorphic effects of the growth-regulator observed in the terminal bud developed also in the axillary buds. By the seventh day on some of the axillary shoots at the second node, the first trifoliate leaves were epinastic; second trifoliates showed similar modification a few days later. Axillary buds at the third and fourth nodes were also strongly affected, often forming rosettes of small, epinastic, silver-haired leaves. In some plants abscission of axillary shoots commenced on the twelfth day. Loss of the main axillary growth at the third node nearly always occurred (fig. 10), and abscission of one of the shoots at the second node was frequent. Characteristically, abscission varied from plant to plant.

In addition to these modifications of vegetative growth, flowering of the treated plants considerably preceded that of the controls. This may be explained in part by the direction of growth from the vegetatively vigorous main axis of the controls to the shorter-lived and earlier-flowering axillary shoots of the treated plants. The morphological distribution and development of flower buds appeared, however, to be the same in both groups of plants. Because of the considerable modification of vegetative growth in treated plants compared with control plants, it was difficult to determine the proportionate production of pods in the two sets of plants. No readily appreciable difference was apparent.

SPRAYING.—The application of the ammonium salt of 2,3,5-triiodobenzoic acid in a spray resulted in a response similar to but much more intense than that exhibited in the ringing experiments. In the garden the substance was applied over significantly larger areas of the

plant, especially in those older plants grown for 27 days before spraying. Undoubtedly the actual total dosage per plant was also greater than that applied by ringing. Generally, the emulsion sprays (figs. 2D, 3B) induced stronger responses than the corresponding aqueous sprays (fig. 1C). Of the three concentrations, the 1.0% spray (fig. 2D) resulted in markedly stronger teleomorphic effects than did the 0.1% spray (fig. 2C). Response to the 0.5% concentration of the ammonium salt was intermediate and difficult to distinguish from either the lower or the higher concentration. Accordingly, discussion will be confined in general to the highest and lowest concentrations.

At the end of 24 hours after spraying, rusty spots were apparent on the leaves in both the young and the old plants. These contact burns were the same color as that which developed over the surface of application in the decapitated and ringed second internodes in the greenhouse experiments. The discolored areas increased slightly for several days but appeared to have little ultimate effect on the growth of the plant. The degree of burning was greater with the emulsion sprays, with the higher concentrations, and in the young stage. Control plants showed no significant effects from spraying either with water or with the emulsion of water and the base mixture.

The epinastic response of leaves (fig. 3A) was more striking in the sprayed plants than in those to which the growth-regulator was applied by ringing. Epinasty of leaflet blades was evident within 24 hours after spraying and grew progressively more severe for several days. By the third day many of the leaflets with inrolled edges and puckered surfaces exhibited as many as two spiral turns in the curled blades. The range in size of the

leaves which responded and the degree of response were also greater in the sprayed plants. The oldest leaves, such as the simple and the lower trifoliate leaves in the old stage of treatment, tended to show but little effect. During the first days after spraying, slight epinasty at the tip of the blades might appear in some of these trifoliates, but by the sixth day the effect was outgrown and the leaf resumed its usual appearance. Less mature leaves in which the length of the blade ranged from approximately 1 to 4 cm. showed the greatest response in rolling and curling of the blades. Expansion of these leaves was also severely checked. Still younger leaves associated with the buds were similarly dwarfed, appeared conspicuously hairy, and exhibited a marked epinastic curling of the tips of the blades. These responses resulted in rosettes of small, curled, silvery leaves about each growing point along the stem.

Abscission of leaves and buds was the dominant characteristic of response to the spray treatment. Among the leaves, those which were most severely affected were the first to abscise. By the third day many of the strongly curled leaflets fell from the plant at the slightest disturbance. Later, progressively more leaf abscission took place. By the tenth day after spraying, especially in the old stage, this was happening down the length of the stem, both to old leaves on the main axis and to younger leaves of the axillary shoot (fig. 2*D*). Although abscission of leaf blades occurs commonly in the bean, it is generally in later stages of maturity of the plant and only of the older leaves.

Even more significant than the response in the leaves was the abscission of buds. The sequence of response appeared to be similar to that observed in the greenhouse. Checking of terminal growth

was evident by the sixth day and was clearly expressed in the formation of rosettes of dwarfed leaves at the growing points. Dulling of the green color and wilting of the dwarfed shoots accompanied suppression of growth. These responses terminated in abscission. By the tenth day after spraying, abscission of the terminal bud had occurred in many of the plants treated in the young stage and was even more frequent in plants of the old stage (table 1).

The continued and progressive action of 2,3,5-triiodobenzoic acid on the growing points of the plant was again exhibited in this experiment. By the tenth day some abscission of axillary buds had taken place in the plants treated in the young stage, and by the fifteenth day this loss of axillary buds was pronounced. In plants of the old stage axillary growth was more advanced at the time of treatment. Response in these growths occurred rapidly so that, by the tenth day after spraying, a large number of axillary buds or shoots had abscised. The physiological action of the growth-regulator persisted and was evident in the response of the accessory buds which developed after the formation of the main axillary shoot. These buds were dwarfed in their development and were successively abscised (table 2; compare results at 40 and 84 days after planting). In many plants this recurring abscission ultimately resulted in a main axis denuded generally of leaves and lateral shoots at the upper nodes (figs. 1*C*, 2*C*, *D*, and 3*C*). Further growth in the denuded plant was directed to shoots at the second node or to buds formed in the axils of the cotyledons at the first node. Growth at the first node was more common than at the second from which buds had frequently abscised. Shoots from these nodes then composed the whole top growth of the plant (figs.

1B, 2C). Generally, these shoots were limited in extent of growth and often continued to show dwarfing (figs. 1C, 2D). In control plants buds at the second node were present but developed only in late stages of maturity, if at all. Formation of a significant part of a control

plant from buds at the cotyledonary node was uncommon.

As a result of successive abscissions, the average total growth of treated plants (figs. 1B, C, 2) was much less than that of untreated control plants (fig. 1A). The severity of reduction in growth

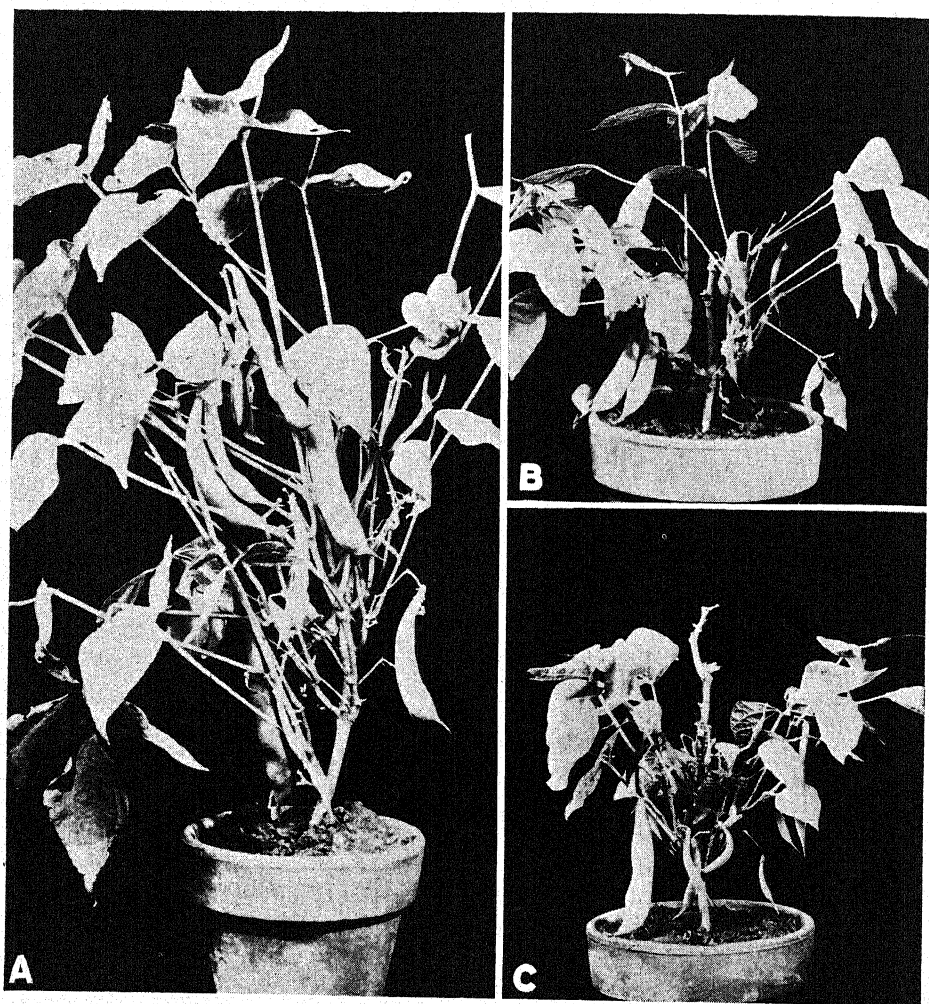


FIG. 1.—A, Red Kidney bean, 77 days after planting, showing development of mature control plant. Note size of plant, well-developed leaves and pods, small shoot at first node, pair of shoots at second node, other large shoots at upper nodes. B, C, 84 days after planting, plants treated with 1% aqueous spray of 2,3,5-triiodobenzoic acid. B, young stage (treated 13 days after planting); C, old stage (treated 27 days after planting). Lateral shoots only at lower nodes, and upper axes denuded; greater dwarfing of plant in old stage. (Figs. 1 and 2, plants grown in garden and transferred to pots; all pictures same magnification.)

varied according to the treatment given (table 3). In general, the highest concentration affected growth more unfavorably than the lowest concentration (fig. 2, *B* and *A*, *D* and *C*). The emulsion sprays resulted in greater reduction in growth than the corresponding aqueous sprays

(figs. 1*C* and 2*D*). Growth from the lower nodes was greater after treatment in the young stage than in the old (fig. 2, *A* and *C*, *B* and *D*). In the severest treatment (1.0% emulsion spray, old stage) some plants did not survive to the end of the period of observation. In the

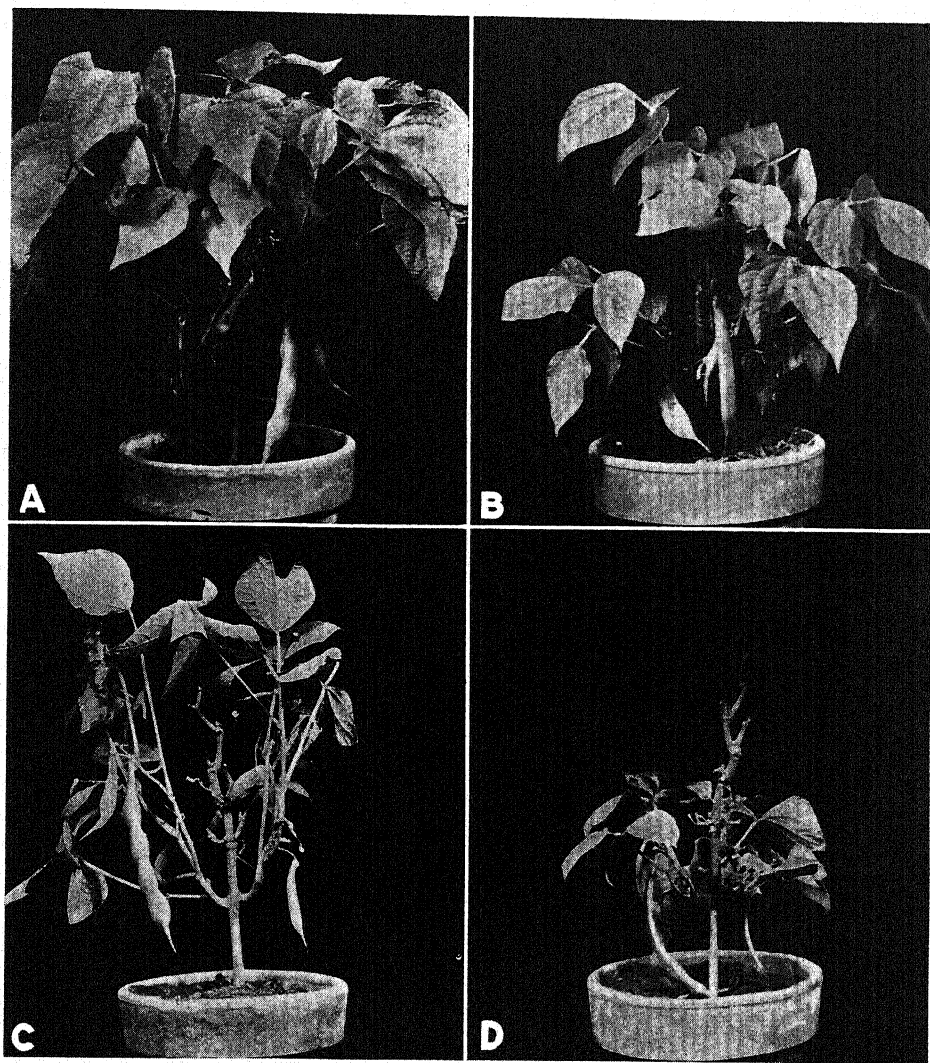


FIG. 2.—Plants treated with various concentrations of emulsion sprays of 2,3,5-triiodobenzoic acid. *A*, 0.1%, *B*, 1.0%, both young stage, 64 days after spraying; *C*, 0.1%, old stage, 49 days after spraying; *D*, 1.0%, old stage, 57 days after spraying. Upper nodes showing stumps of abscised axillary and accessory shoots. More severe effect with old stage and 1.0% concentration; in *C* note major growth of plant at first node.

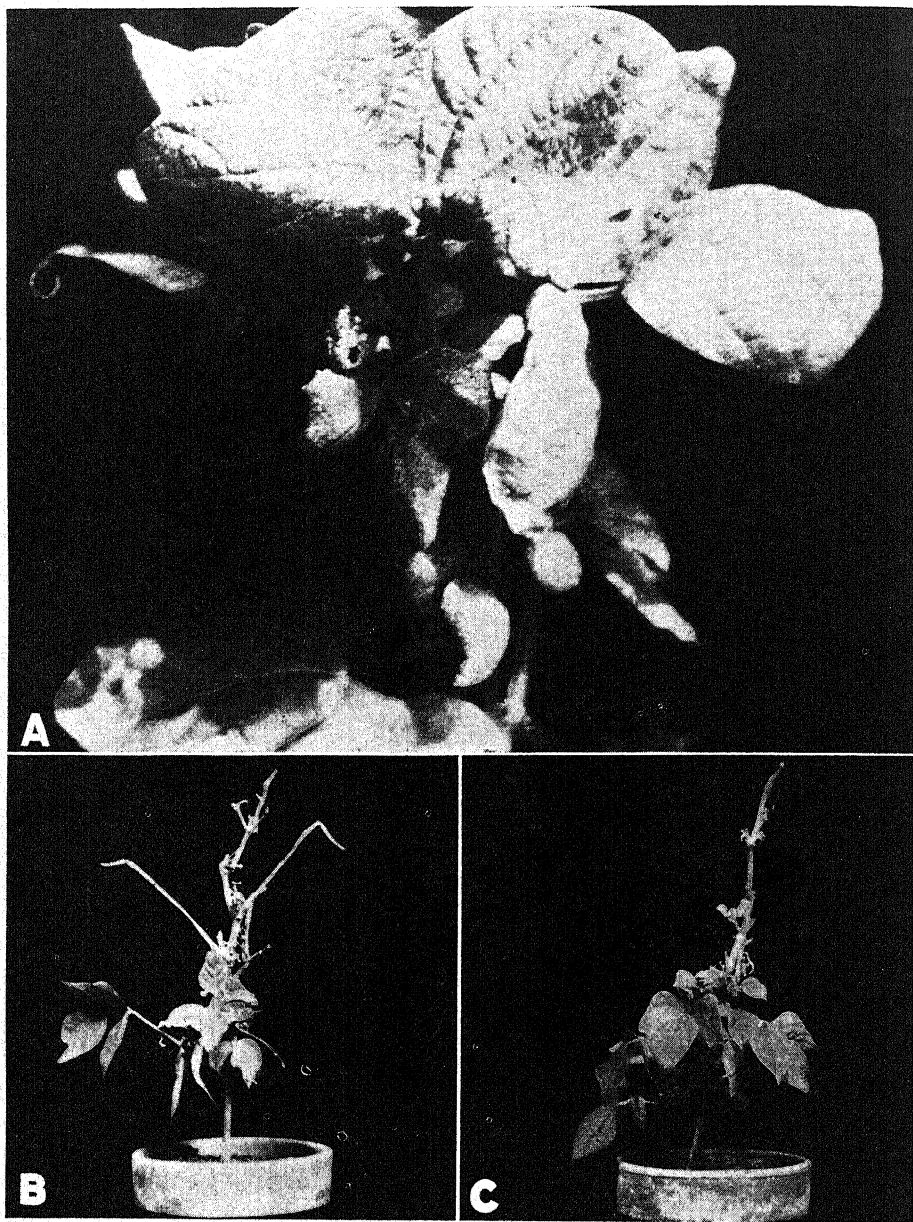


FIG. 3.—Plants treated with emulsion sprays of 2,3,5-triodobenzoic acid, old stage. *A*, 1.0%, 14 days after spraying. Simple leaves showing no effect; slight epinasty in older trifoliate leaves; young leaves dwarfed, severely curled, and with pebbled surfaces. *B*, 1.0%, *C*, 0.5%, both 49 days after spraying. Dwarfed growth at lower nodes; at upper nodes abscission of leaflet blades in *B* and of whole leaves in *C*.

treatment resulting in least reduction (0.1% aqueous spray, young stage) a number of plants showed recovery of the terminal bud and abscission of only a few lateral buds.

Flowering and the formation and maturation of pods occurred in all treatments (figs. 1, 2). No appreciable differences were noted between the young stage in which floral buds were undifferentiated at the time of spraying and the old stage in which floral buds were present. The morphological distribution and form of the flowers and fruit appeared to be unaffected. In many plants on which a profusion of axillary and accessory shoots had developed at lower nodes as a consequence of treatment, the period of flowering was prolonged, and the number of flowers and young fruits was markedly greater than in control plants. Abscission of these flowers and young pods was frequent, although a few pods persisted to maturity. A comparison of fresh weights of plants and pods (table 3) suggests that the primary effect of treat-

ment was on vegetative structures. Modification of the vegetative habit in turn influenced flowering and fruiting; severe reduction in growth significantly reduced

TABLE 1

PERCENTAGE OF BEAN PLANTS SHOWING ABCISSION OF TERMINAL BUD 10 DAYS AFTER TREATMENT WITH SPRAYS OF AMMONIUM SALT OF 2,3,5-TRIODOBENZOIC ACID*

Spray	Concentration (%)	Young stage (23 days after planting)	Old stage (37 days after planting)
Emulsion...	1.0	47	89
	0.1	76	85
Water.....	1.0	54	93
	0.1	36	81

* Analysis based on twenty or more plants per treatment.

pod production; less severe effects resulted in less appreciable differences in pod production. The high degree of modification of the vegetative growth, however, presented such serious difficulties in interpretation of the flowering habit that observations are inconclusive.

TABLE 2

ABSCISSION OF LATERAL BUDS OR SHOOTS IN BEAN PLANTS TREATED WITH EMULSION (E) AND AQUEOUS (W) SPRAYS OF AMMONIUM SALT OF 2,3,5-TRIODOBENZOIC ACID*

A=developing axillary bud or shoot
a=developing accessory bud or shoot

O=abscised axillary bud or shoot
o=abscised accessory bud or shoot

TYPE OF LEAF	NODES OF MAIN AXIS	40 DAYS AFTER PLANTING						84 DAYS AFTER PLANTING					
		Young stage (27 days after spraying)		Old stage (13 days after spraying)		Control		Young stage (71 days after spraying)		Old stage (57 days after spraying)		Control	
		1.0% E	0.1% W	1.0% E	0.1% W			1.0% E	0.1% W	1.0% E	0.1% W		
Trifoliates	10	Aa	Aa
	9	Oa	Oa	Aa	O	O
	8	Oa	aOa	Aa	O	aA
	7	Oa	aOa	Aa	O	aA
	6	aOa	Oa	aAa	O	oOo	aAa
	5	A	aOa	A	oOo	oOo	aO
	4	aOa	aAa	A	aOa	A	oOo	Oa	oO	Oo	Aa
Simple Cotyledons	3	aOa	aAa	A	aOa	Aa	oOo	aO	Oo	oOa	oAa
	2	aOaaOa	aOa Aa	aOaaOa	aOaaOa	A A	oOooOo	aAaaAo	oOaaOo	aOaaOa	OoAo
	1	A O	A A	A A	A A	A A	O O	A A	A A

* Analysis based on representative plant for each group; variation from plant to plant to be expected.

HISTOLOGICAL RESPONSES

DECAPITATION.—The initial effect of application of 2,3,5-triiodobenzoic acid in lanolin to the decapitated second internode was killing at the cut surface. Necrosis was observed across all tissues immediately at the surface and downward along the epidermis and into the cortex at points where the lanolin paste was apparently in contact with the exterior of the stem. Deeper penetration of the killing effect occurred for several days

killing effects at the uppermost levels. Cells of the inner cortical parenchyma began to proliferate after initiation of activity in the endodermis. At first only those cells adjacent to the endodermis responded with one or two divisions (fig. 6*B*). In later stages and at the uppermost levels, however, more of the outer cells became active, and divisions were more numerous (figs. 5*B*, 7*B*).

Proliferation of endodermal cells was the first readily noted histological re-

TABLE 3

AVERAGE* FRESH WEIGHTS OF SHOOTS AND PODS OF BEAN PLANTS, 79 DAYS AFTER PLANTING, TREATED WITH SPRAYS OF AMMONIUM SALT OF 2,3,5-TRIIODOBENZOIC ACID

PLANTS	CONC. OF SPRAY (%)	WEIGHT OF WHOLE SHOOT† PER PLANT (GM.)		WEIGHT OF PODS PER PLANT (GM.)	
		Emulsion	Water	Emulsion	Water
Young stage (66 days after spraying)	1.0	21.7	31.5	4.5	13.0
	0.1	32.3	62.9	11.3	24.5
	Control	65.3	62.2	23.8	23.2
Old stage (52 days after spraying)	1.0	18.8	38.0	4.1	15.1
	0.1	23.1	51.7	6.3	23.5
	Control	82.3	71.6	31.8	28.2

* Averages based on ten plants per treatment.

† Roots removed at ground line.

after application. Downward extensions in the pericycle, the secondary phloem, xylem elements, and in the pith were noted. The killed tissues stained dark red, and those at the surface collapsed. Tissues below the level of killing began to proliferate rather slowly as compared with the response to many other growth-regulating substances. A variability in rate of response was encountered in all collections and correlated with gross observations already noted. The pattern of proliferation within the various tissues, however, seemed to be consistent.

In the epidermis little or no response to treatment was noted apart from the

sponse to treatment (fig. 4*A*). By the third day initial divisions were evident in the endodermis in some stems, while in other stems no proliferation was as yet apparent. Within the next few days proliferation became well established, although the rate varied considerably from stem to stem and even within the sectors of a stem. The band of derivatives frequently retained the pattern of rows resulting from successive tangential divisions (figs. 5*B*, 6*B*). In areas of greater activity, however, this order was lost (fig. 7*B*). Maturation of wound tracheids commenced among the inner endodermal derivatives as early as 7 days after de-

capitation. As maturation continued (figs. 5*B*, 7*B*), more of these short tracheids were differentiated in groups or sometimes in strands which merged with similar structures in the rays. As late as 25 days after application, organization of the tracheids into clearly defined vascular bundles was not evident, although some of the derivatives of the endodermis were still active. Maximum proliferation (fig. 7*B*) occurred about the 1 mm. level below the cut surface. Downward the band of derivatives quickly narrowed (fig. 8*A*) so that between 2 and 3 mm. the single-layered endodermis was present (fig. 8*B*).

The pericycle (fig. 5*A*) was more subject to the killing effects of the growth-regulator than most of the other tissues, except perhaps the pith. At the cut surface and down 1 mm. or more, these elongated cells showed the dark-red color indicative of killing. At lower levels the cells remained inactive and were frequently displaced by proliferation of adjacent tissues.

Phloem parenchyma, both primary and secondary, became active somewhat later than the endodermis. Proliferation was initiated about the fifth day and was well advanced by the seventh (fig. 6*B*). Divisions were in various planes so that no distinctive organization of derivatives developed (fig. 4*B*). Maturation was similar to that in the endodermis. Wound tracheids were formed in patches and strands (figs. 5*B*, 7*B*). Meanwhile, other derivatives continued to proliferate, often crushing the sieve tubes and companion cells which apparently remained inactive. These inactive elements, like the pericyclic cells, often exhibited traces of the downward killing effect.

Probably the earliest response occurred in the cambium. By the fifth day

cambial activity had resulted in a wide band of undifferentiated derivatives. Maturation commenced with the differentiation of the innermost derivatives as secondary tracheids (fig. 6*A*). In some stems this began as early as the fifth day. The band of tracheids, intermingled with parenchymatous cells, continued to widen for several days. Maximum proliferation was about 1 mm. below the cut surface. At successively lower levels, the degree of cambial proliferation gradually decreased. At levels below any apparent response, the formation of the usual secondary xylem tracheids or fibers had scarcely begun (fig. 8*B*). Lignified xylem elements were unresponsive. During the first week after application xylem parenchyma cells possessed markedly dense contents. By the seventh day divisions commenced in some of these parenchymatous cells. Proliferation continued and resulted in a mass of small cells about the vessels (figs. 6*B*, 7*B*). This development was observed only at the uppermost levels.

In the rays proliferation in the outer portion was similar to that in the phloem or endodermis. Likewise tracheids were matured (fig. 7*A*), often as strands connecting tracheids in the endodermis with those derived from the cambium. In the inner portion of the ray some of the parenchyma cells adjacent to the xylem underwent a few divisions, commencing about the twelfth day. The pith was inactive and showed the deepest general penetration of the killing effects of 2,3,5-triiodobenzoic acid.

RINGING.—In the ringing experiment histological response at the point of application consisted of contact killing similar to that observed at the cut surface in decapitation. Injury of the tissues occurred most commonly along the ridges over the large vascular bundles of

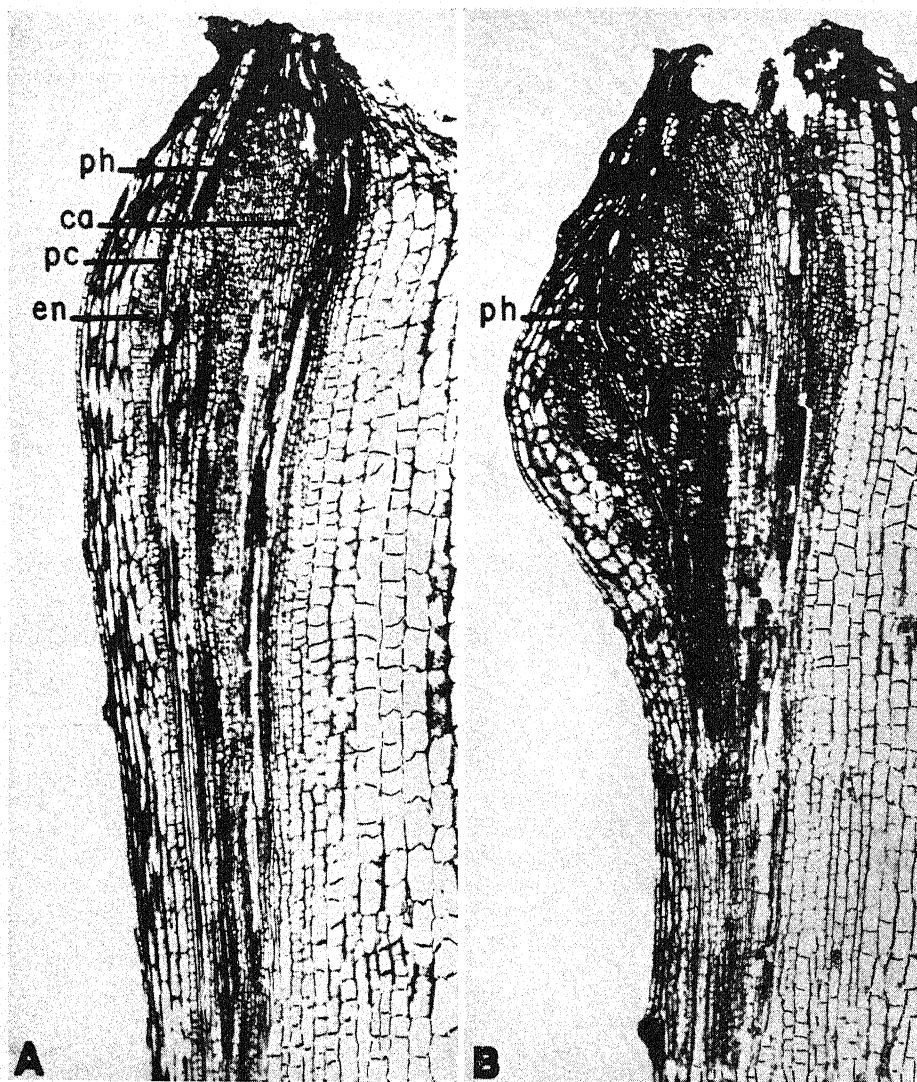


FIG. 4.—Longisections of decapitated stems treated with 2% 2,3,5-triiodobenzoic acid in lanolin. *A*, 5 days after application. Killing of tissues at cut surface with traces of killing downward in pericycle (*pc*), phloem (*ph*), and pith; early proliferation in endodermis (*en*) and in cambial zone (*ca*). *B*, 12 days. Marked proliferation in phloem and continued activity in endodermis and cambial zone.

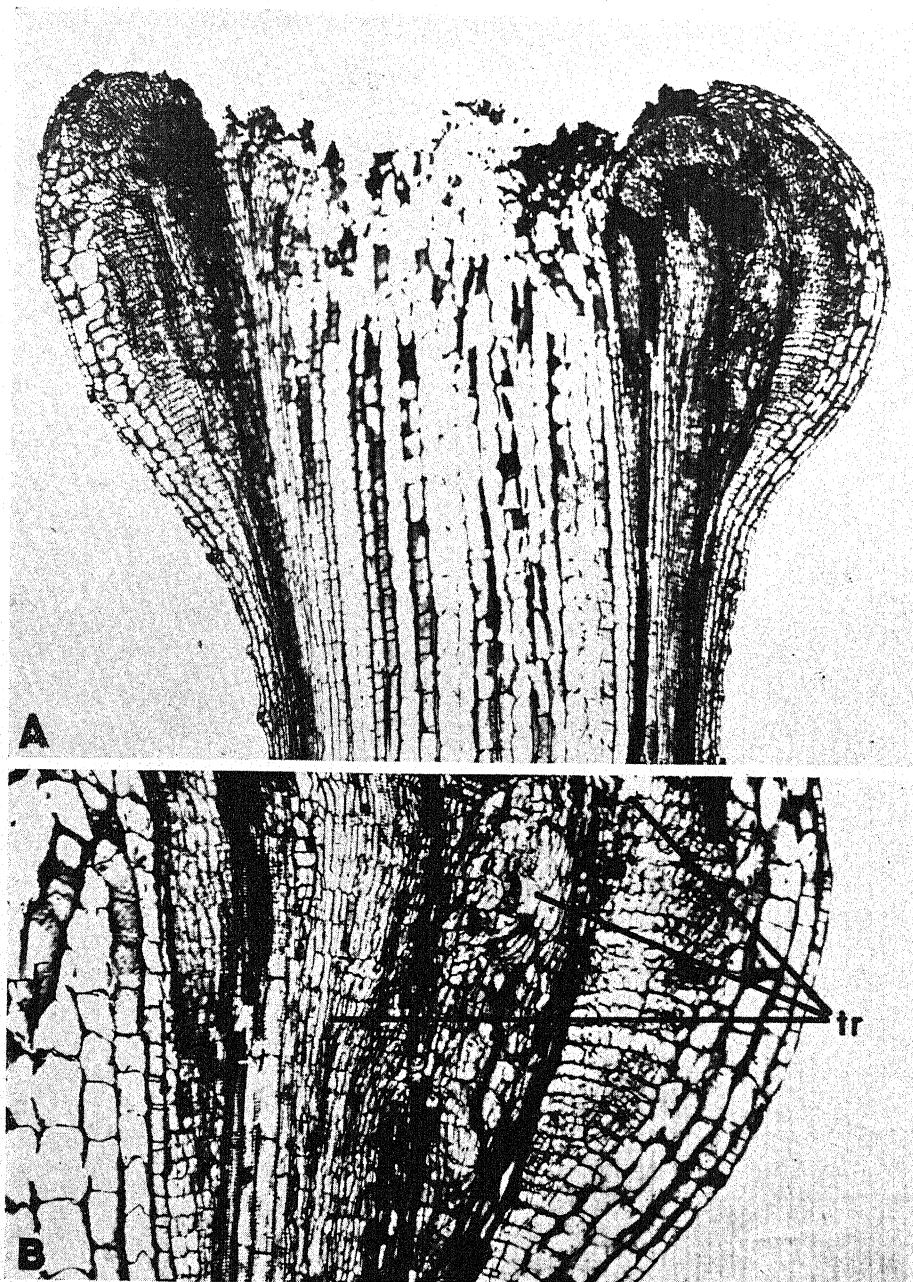


FIG. 5.—Longisections of tumor, 25 days after application. *A*, killed tissues at surface disintegrating; major proliferation approximately 1 mm. below cut surface; cortical parenchyma active at uppermost levels. *B*, detail of *A*. Proliferation greatest in endodermis, phloem, and cambial zone; maturation of many tracheids (*tr*) in proliferated tissues; outer cortical parenchyma and pericycle inactive.

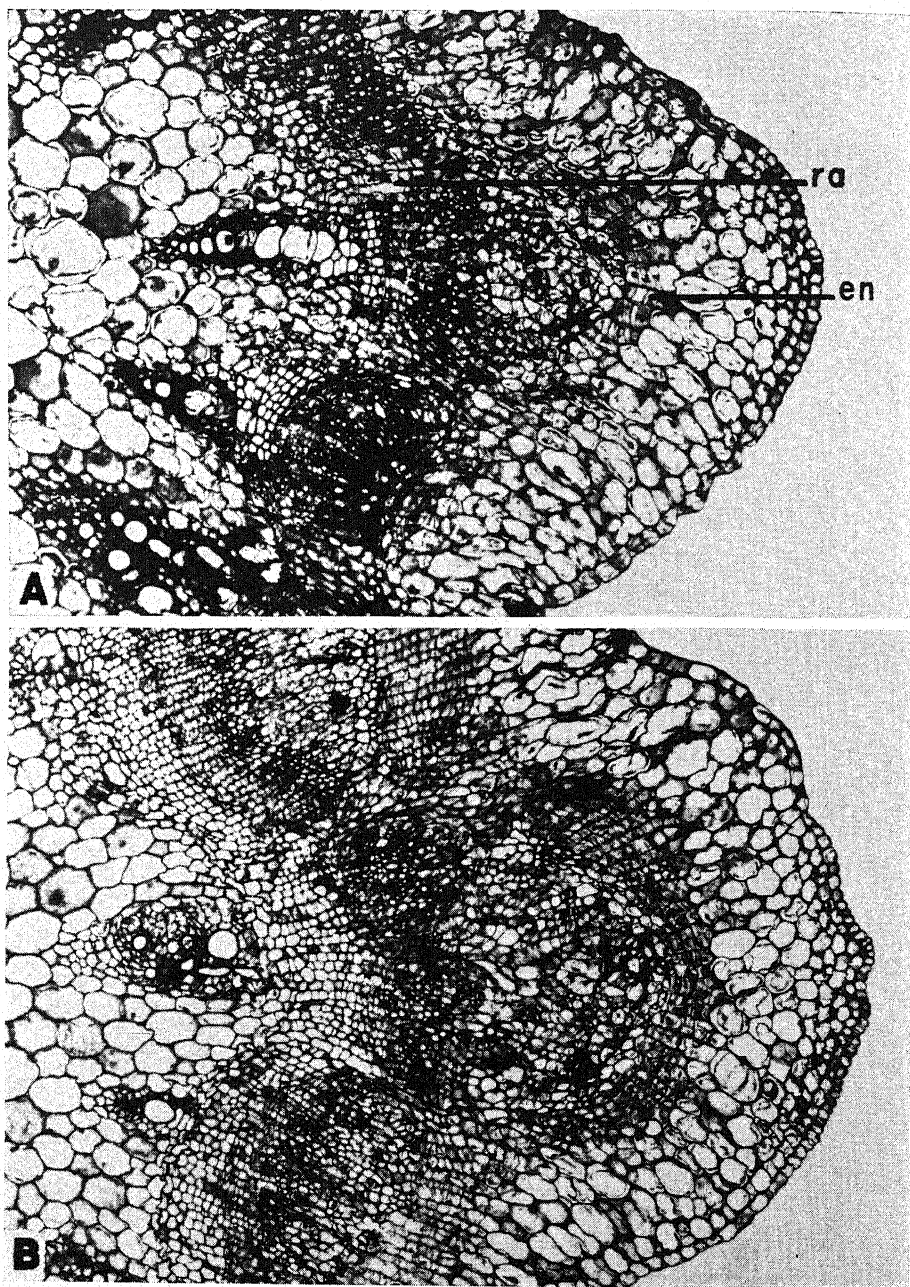


FIG. 6.—Transections of tumors approximately 1 mm. below cut surface. *A*, 5 days. Early proliferation in endodermis (*en*) and outer portion of ray (*ra*); initial activity in phloem; maturation of tracheids from cambial derivatives. *B*, 7 days. Proliferation in endodermis and phloem well established; band of tracheids matured from cambial derivatives; proliferation of xylem parenchyma.

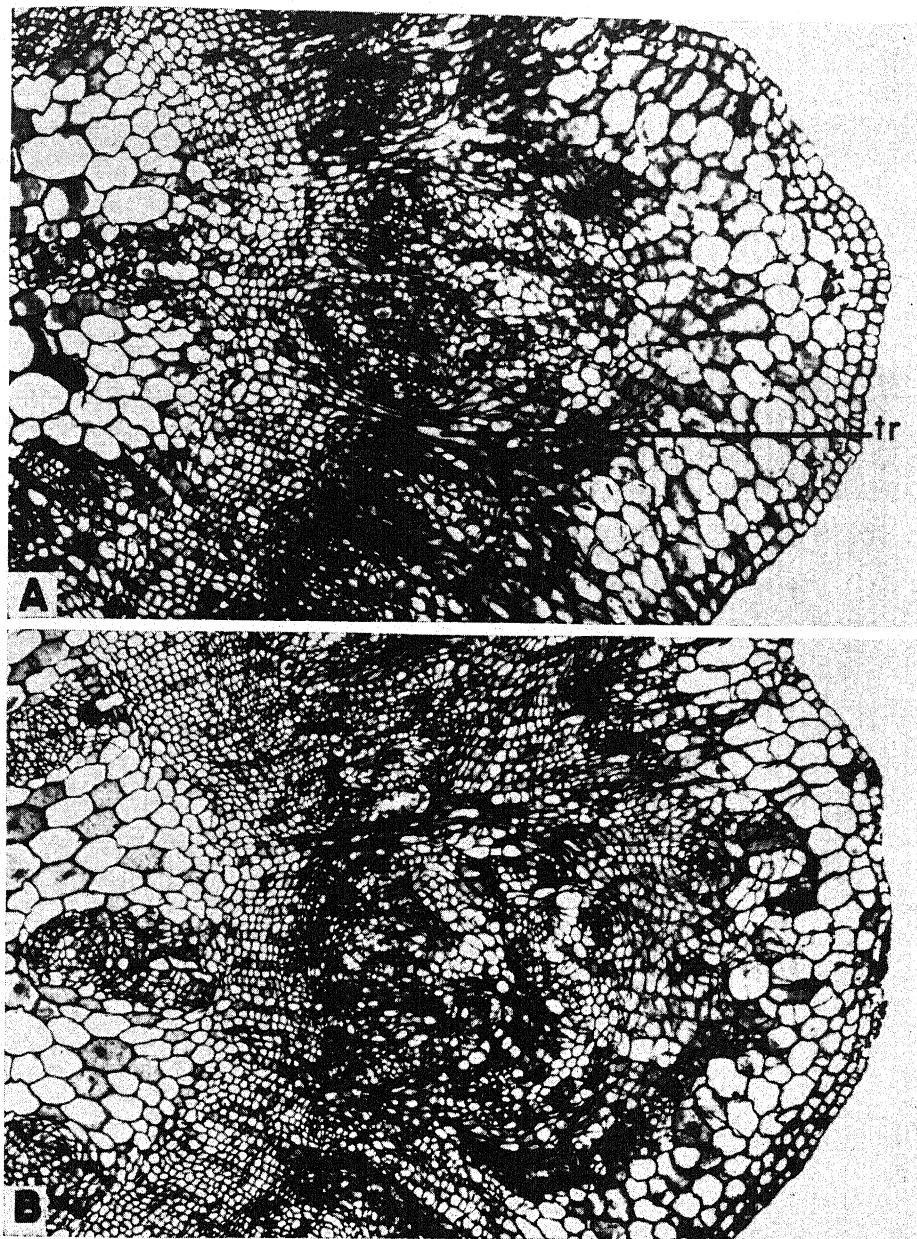


FIG. 7.—Transsections of tumors approximately 0.5 mm. below cut surface. *A*, 12 days. Continued maturation; strands of tracheids (*tr*) matured in rays and connecting endodermal tracheids with xylem tracheids. *B*, 25 days. Proliferated tissues matured except for small areas of continued activity. Note extent of proliferation in inner cortical parenchyma.

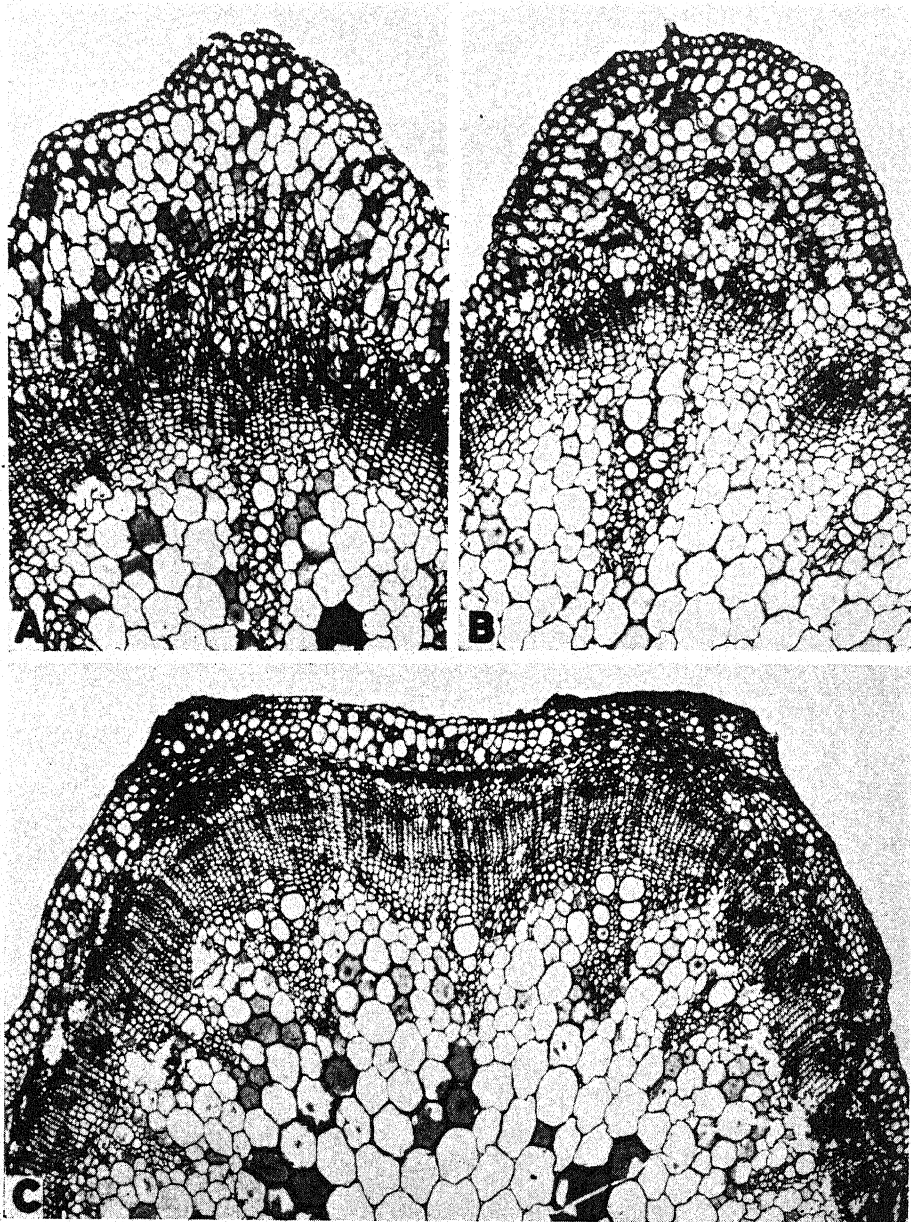


FIG. 8.—*A, B*, transections of same tumor as fig. 7*B*. Traces of proliferation 1.5 mm. (*A*) and no apparent response at 3 mm. (*B*). *C*, transection of second internode at level of ringing with 2% 2,3,5-triiodobenzoic acid in lanolin, 15 days after application. Killing of outer tissues over large vascular bundles. Note amount of secondary tissues derived from cambium.

the stem (fig. 8C). Cells lying in the area extending from the epidermis to the outer portions of the phloem exhibited the dark-red color characteristic of killed cells. Proliferation in these tissues was not characteristic, although increased activity of the cambium might occur.

In addition to observations in the area of ringing, histological examinations were made of structures exhibiting teleomorphic responses. Although no collections were made of the plants sprayed in the garden, gross responses were so similar to those observed in the greenhouse ringing experiment that essentially the same anatomical changes probably occurred in both experiments.

Of the teleomorphic responses induced in treated plants, the loss of plant parts was the most significant. Histological study of a series of stem tips of plants treated in the ringing experiment disclosed that the loss of buds and leaves was consequent to a well-defined abscission process. Furthermore, several abscission layers might be induced in one stem (fig. 9A, B). Abscission layers were found generally at midlevel in the young internodes immediately below the terminal bud. In the young plants treated in this experiment these were the third, fourth, and fifth internodes. In some plants abscission layers were found in all three of these internodes (fig. 9A); in other plants only one or two layers were observed. The internode in each case appeared to be only partially elongated with the xylem matured to the extent of lignification of the primary elements. Similar abscission layers were observed in axillary stems (figs. 9B, 10). Leaf fall was likewise the result of separation in a definite abscission layer which was formed at the base of the petiole (fig. 9A).

Abscission appeared to be of the type

commonly found in herbaceous plants (2) in which a simple separation layer is formed shortly before abscission. Changes in stem structure were noted by the sixth or seventh day after ringing. One or more layers of parenchymatous cells across the internode divided horizontally to form a band of cells flatter and smaller than those of adjoining areas and some eight or more cells deep (fig. 11A). Cells in tissues below this separation layer continued to enlarge, whereas growth in tissues above the layer appeared to be suppressed (fig. 11B). In the growing plant the dwarfing, discoloration, and wilting of the bud and associated leaves were undoubtedly correlated with this development. Abscission occurred in the upper portion of the separation layer (fig. 12A), and final loss of the bud was readily brought about by any mechanical disturbance to the plant. In the greenhouse loss of terminal buds began as early as 9 days after treatment. Following abscission the outer cells of the remaining tissues became lignosubersized to form a protective layer (figs. 10, 12B).

Histological examination of leaves was limited. Suppression of growth was evident in the closely aligned, small, rectangular cells of the mesophyll and in the absence of the characteristic intercellular spaces (fig. 13B, C). The epinastic curling response of the blade (fig. 13A) was not the consequence of appreciable hypertrophy of any given tissue. Loss of leaves followed the formation of abscission layers as already noted (fig. 9A).

Discussion

In comparison with tumors induced by many other growth-regulating substances, tumors formed in response to 2,3,5-triiodobenzoic acid were relatively insignificant in size, and the degree and

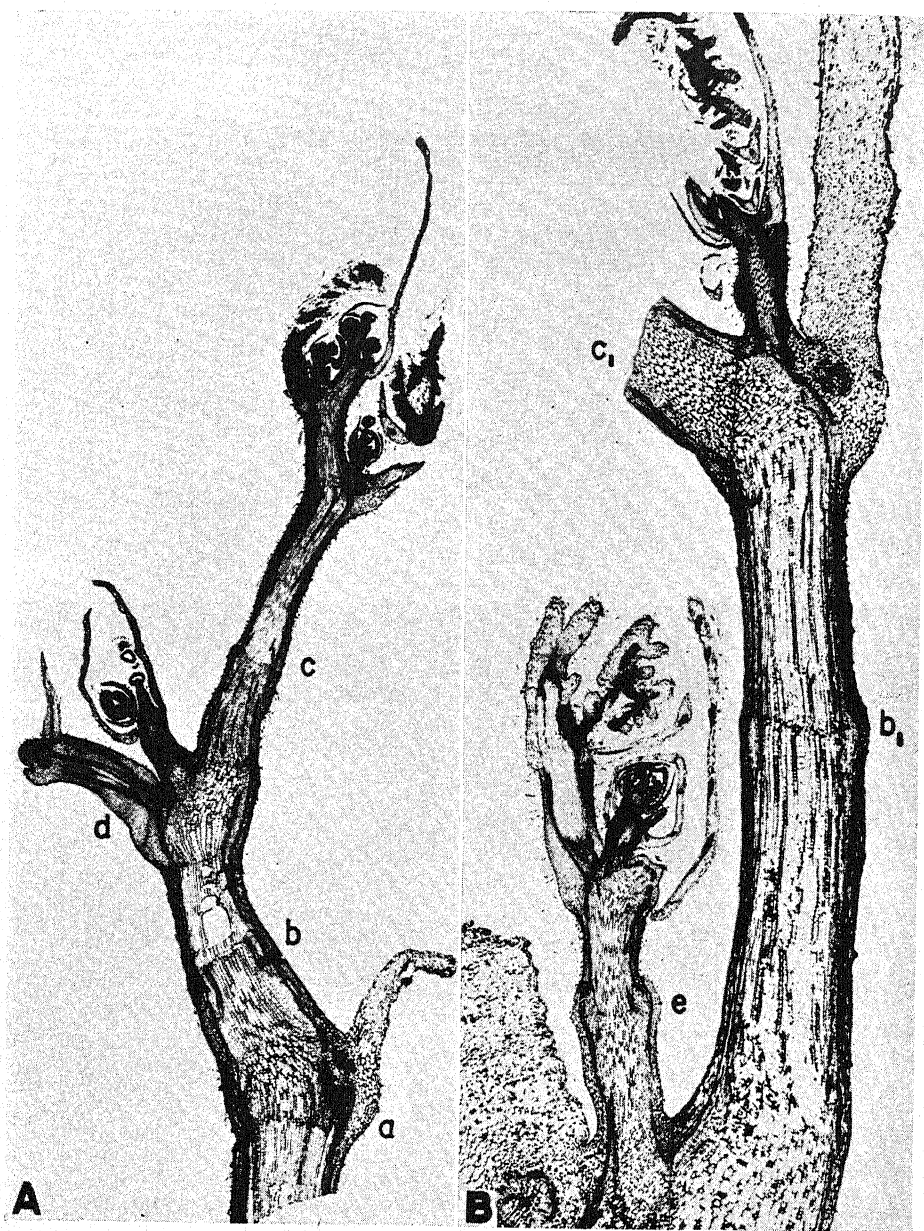


FIG. 9.—Reconstructions in longisection of terminal portions of two stems. *A*, 7 days and *B*, 9 days after ringing second internode with 2% 2,3,5-triiodobenzoic acid in lanolin. Abscission layers formed at second (*a*), third (*b*, *b*₁), and fourth (*c*) internodes of main axis; in axillary branch (*e*); at base of second trifoliate leaf (*d*). Terminal bud abscised in fourth internode (*c*₁).

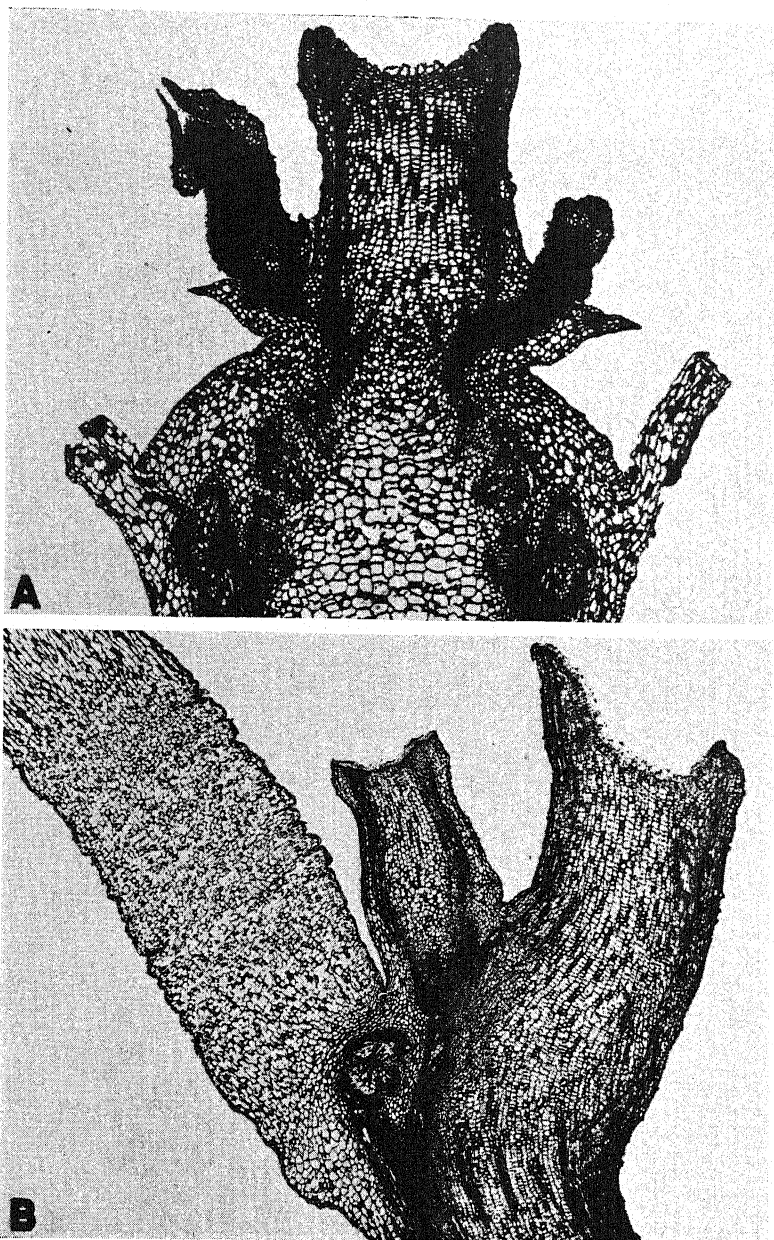


FIG. 10.—Longisections at third node of main stem; plants treated by ringing. *A*, 12 days after ringing. Section through axil showing abscised axillary shoot and two lateral, accessory buds. *B*, 18 days after ringing. Section showing abscised axillary branch (*center*) in axil between first trifoliate leaf (*left*) and main stem (*right*). Main stem abscised in middle of third internode.

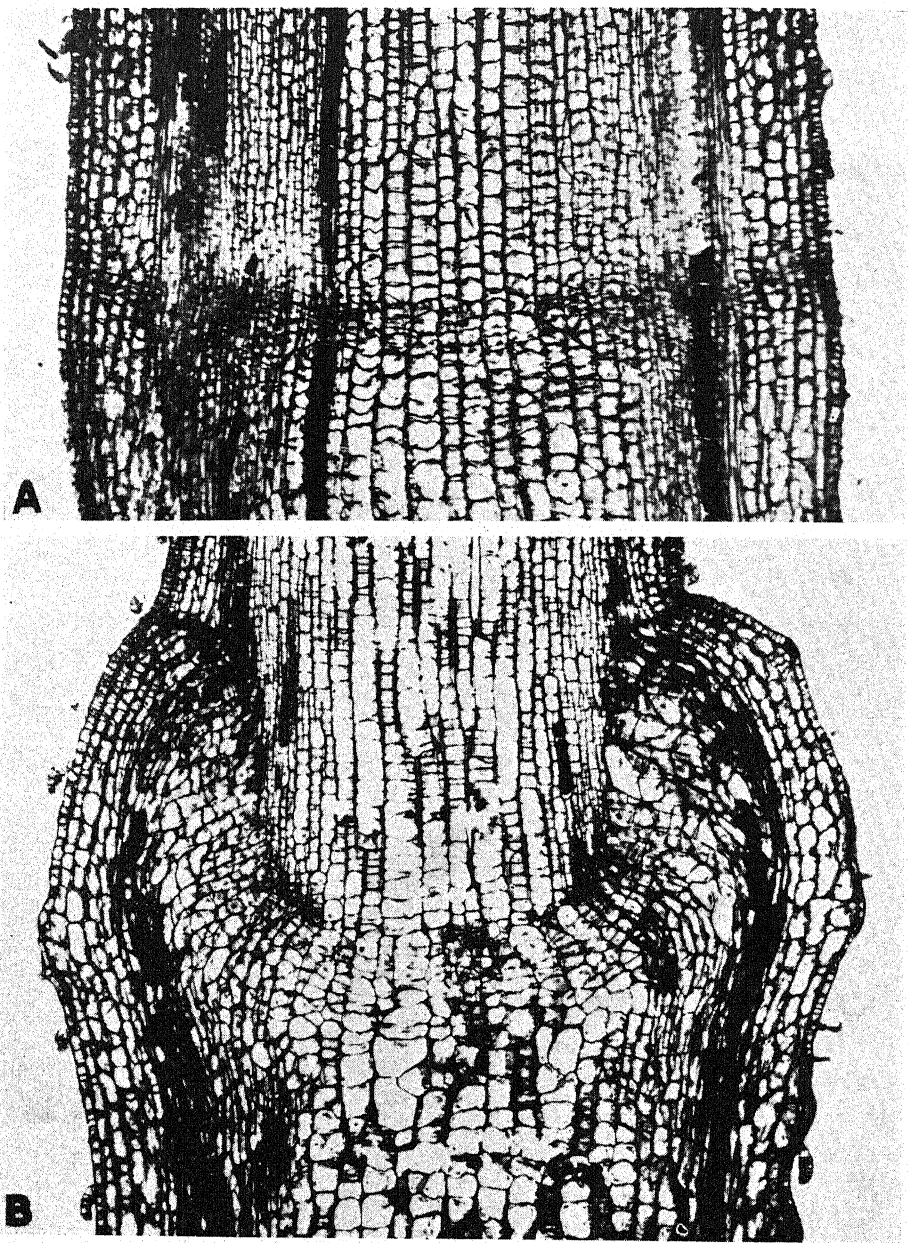


FIG. 11.—Longisections showing early stages in abscission of axillary branch formed at third node. *A*, 7 days after ringing. Initial cell divisions in development of separation layer across parenchymatous tissues of stem. *B*, 9 days. Well-developed separation layer extending across stem. Enlargement of stem below layer and suppression of growth above layer indicated by differences in cell size. (Detail of abscission layer *c* in fig. 9*B*.)

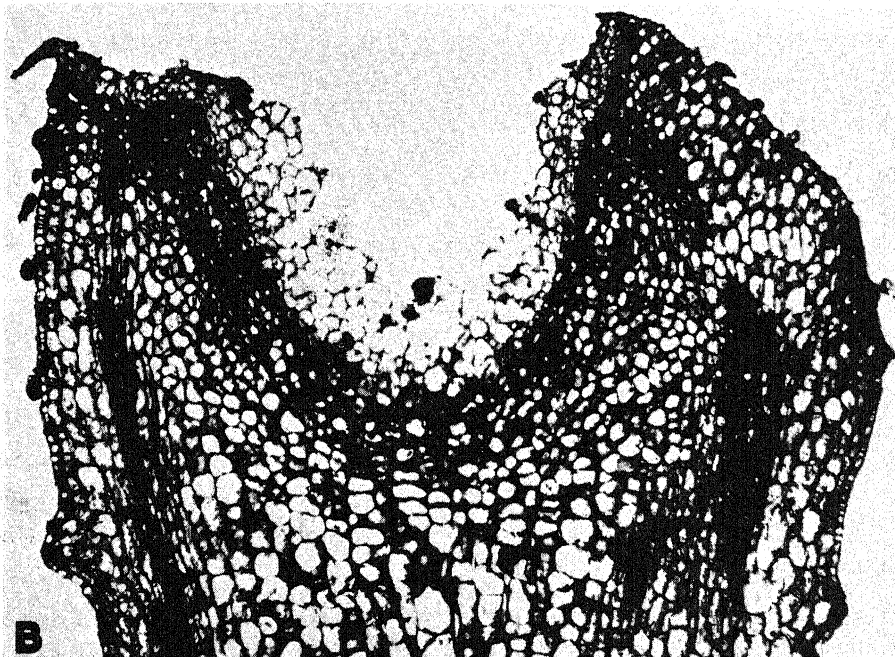
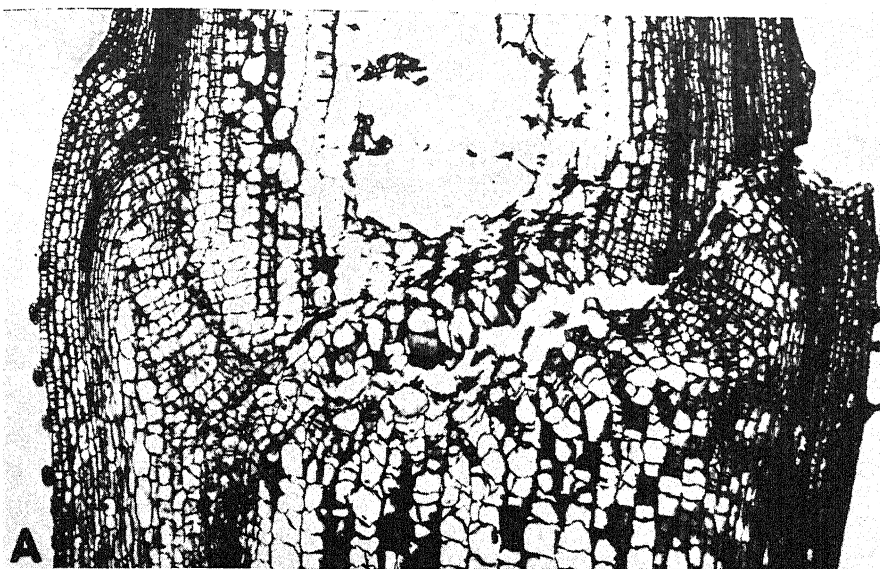


FIG. 12.—Longisections showing late stages in abscission. *A*, 12 days after ringing; section of third internode. Abscission commencing in upper portion of separation layer. *B*, 18 days; section of axillary branch developed at third node. Abscission completed; outer cells forming lignosuberized protective layer.

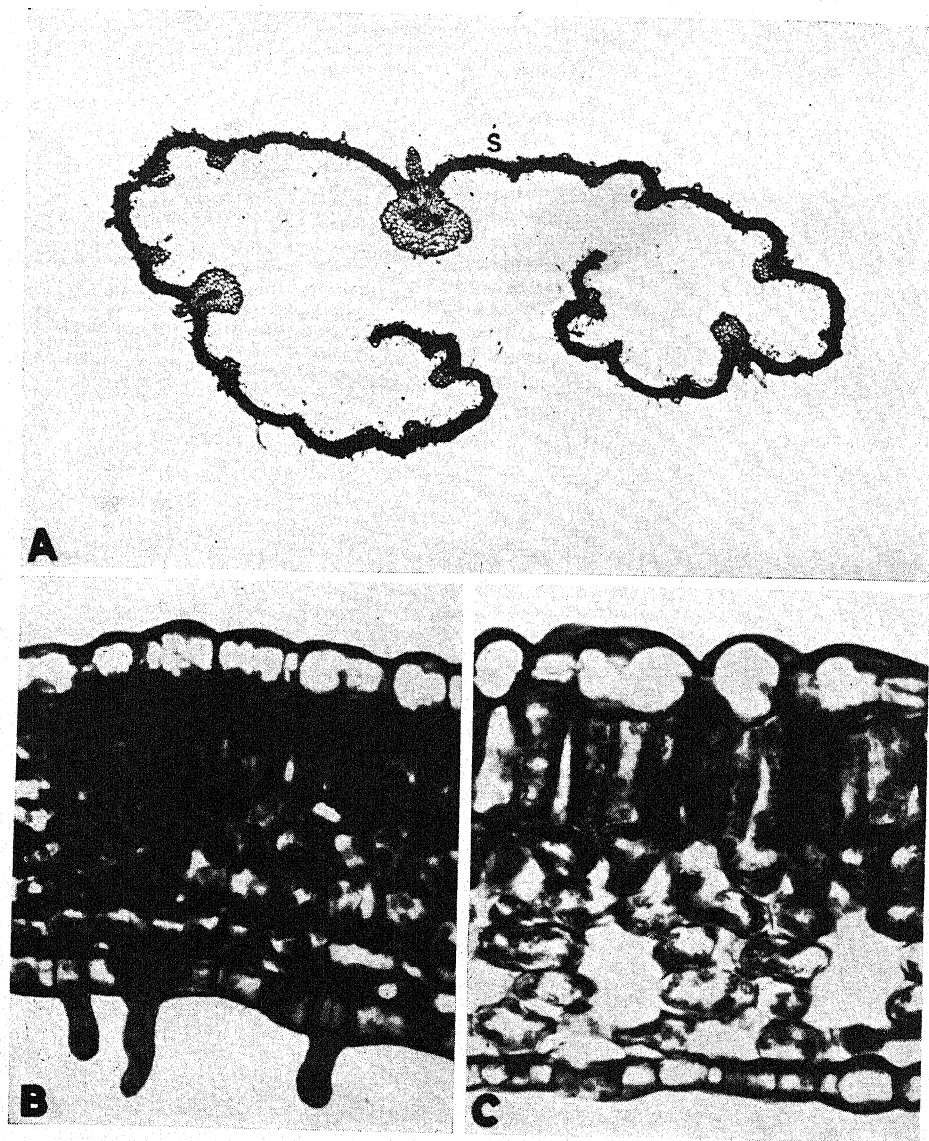


FIG. 13.—Sections of young leaflet blades. *A*, entire blade from plant treated by ringing. *B*, detail of *A* at position *s*. *C*, detail of blade from control plant, section from position similar to *s*. Note epinastic response in rolled margins of *A*. Compare compactness and arrangement of cells and development of intercellular spaces in *B* and *C*.

elaboration of tissue proliferation were limited. The tissues which proliferated were the same as those which have responded to other growth-regulators; however, the total pattern of development in these tumors differed from the pattern induced by other growth substances and by wound response (11). Although application of both 2,3,5-triiodobenzoic acid and 2,4-dichlorophenoxyacetic acid result in teleomorphic responses, there was little resemblance between tumors induced by the two substances with the same experimental methods (8). Distinguishing characteristics of tumors induced by 2,3,5-triiodobenzoic acid are: proliferation of the endodermis, the phloem parenchyma, and the cambium; maturation of many derivatives as tracheids; lack of organized vascular bundles in the proliferated tissues; and absence of root primordia.

The abscission of leaves and buds following the formation of a well-defined separation layer appears to be the significant teleomorphic response to 2,3,5-triiodobenzoic acid. The significance of this response is emphasized by the persistence of the effect of the compound in inducing progressive abscission of newly formed buds and leaves. This ability to induce abscission contrasts sharply with the action of many other growth-regulating substances in delaying or inhibiting abscission. The widespread use of growth substances in preventing preharvest drop of apple and other fruits depends on this inhibiting action of naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid, and other substances. Histological evidence of the inhibition of abscission by a growth-regulating substance has been reported (1). Plants of *Mirabilis jalapa* decapitated in the first internode and given no further treatment showed cessation of growth in the inter-

node followed by abscission at a well-formed separation layer. Decapitated first internodes treated with 2% indoleacetic acid failed to abscise, and the abscission zone was altogether absent. Other investigators (4, 7, 9) have studied the effects of auxin, indoleacetic acid, and various other substances in delaying the abscission of leaves of *Coleus*. In each case the availability of a source of auxin or growth substance is associated with the delay in abscission. This suggests, conversely, that reduction in auxin levels may be associated with the induction of abscission. Inasmuch as anti-auxinic activity has been attributed to 2,3,5-triiodobenzoic acid (3), abscission appears to be a not unreasonable sequence to treatment with this substance.

GALSTON (3) found indications of auxin aberrations and lowering of auxin levels at meristems in treated soybean plants. Likewise in the present experiments, the teleomorphic responses occurred mainly at the growing points and in associated young organs. That translocation of this substance to the buds may occur is supported by the work of WOOD *et al.* (12). These investigators used a radioactive plant growth-regulator (2-iodo-3-nitrobenzoic acid) which induced responses similar to those elicited by 2,3,5-triiodobenzoic acid. Radioactivity measurements indicated that the major accumulation of the substance occurred in the terminal buds of treated bean plants. If the action of 2,3,5-triiodobenzoic acid is mainly at points of accumulation and results in lowered auxin levels and if auxin reduction is a factor in inducing abscission, then the abscission of buds and leaves in response to treatment with 2,3,5-triiodobenzoic acid is consistent.

2,3,5-Triiodobenzoic acid has aroused interest as a growth-regulator because of

reported florigenic activity (14). Recent experiments have not presented conclusive results on this problem. GALSTON (3) considered that it did not possess florigenic properties in itself but might influence flowering because of its action on auxin levels. Differences in the character of response according to the state of flowering at the time of treatment have also been reported (3, 10). In the present experiment, however, modification of the flowering habit appeared to be correlated with modification of the vegetative growth. No appreciable differences in the number of flowers and pods resulted from spraying the plants in two different stages of floral bud development. Likewise, there was no evidence of independent, florigenic action of 2,3,5-triiodobenzoic acid in altering either the form or the distribution of the flowers of the bean plant.

Summary

1. Small tumors were formed at stem tips of Red Kidney bean plants decapitated in the second internode and treated with a 2% concentration of 2,3,5-triiodobenzoic acid in lanolin. Proliferation occurred mainly in the endodermis, phloem parenchyma, and cambium. Many derivatives matured as tracheids. Neither vascularization of the tumor nor formation of root primordia took place.

2. Teleomorphic responses resulted from treatment with 2,3,5-triiodobenzoic acid in a 2% concentration in lanolin paste applied in a ring around the middle of the second internode of young plants. Similar teleomorphic responses followed application of the ammonium salt of this acid in a spray. Three concentrations of the salt (1.0, 0.5, and 0.1%) were used, each in an aqueous and in an emulsion spray. Plants were treated in a young and an old stage.

3. The most significant teleomorphic response was the abscission of leaves and buds following the formation of separation layers induced by treatment. Persistence of the effect of the substance was evident in the progressive abscission of the new growth. Epinasty of leaves and dwarfing of growth also occurred.

4. Apart from considerable modification of vegetative growth, treatment with 2,3,5-triiodobenzoic acid appeared to have no independent effect on floral morphology or distribution under conditions of the present experiments.

5. The induction of abscission by this compound is discussed in relation to the role of auxin and the inhibition of abscission by many plant growth-regulating substances.

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RESPONSES OF CROP PLANTS TO O-ISOPROPYL N-PHENYL CARBAMATE¹

W. B. ENNIS, JR.²

Introduction

The effects of O-isopropyl N-phenyl carbamate upon several species of plants have been reported (1, 2, 3, 4, 5, 7, 8, 9). Although certain features of the external responses to its application in these few plants have been described, the published information is far from complete and little is known as to the symptomatic responses of many other plant species to this substance. TEMPLEMAN and SEXTON (9) first observed an apparent differential response of cereals and certain dicotyledonous species following treatment with this compound and found that it was more effective in inhibiting cereal growth than were several other urethanes. They described the arrestment of growth and the development of a thickening of the basal region of oat plants. Charlock, on the other hand, seemed unaffected. ALLARD *et al.* (2) described this same response in oats and also a characteristic dark blue-green

coloration of the leaves of cereal plants treated with this carbamate. They also reported that certain dicotyledonous plants were not affected.

CARLSON (3) found that the carbamate was effective in "destroying rhizomes of quack grass." MITCHELL and MARTH (7) reported that it was active in preventing germination of *Agropyron repens* L. but that certain dicotyledonous plants were not affected by similar treatments. TAYLOR (8) did not observe any influence of this substance upon dicotyledonous weeds and only a slight inhibitory effect on weedy grasses. ENNIS (5) outlined the gross responses of *Avena* and certain other species to this compound and described the cytological and histological reactions to it. From these various studies, it is obvious that the responses of plants to this substance are distinctly different from those induced by the halogen-substituted phenoxyacetic acid type of plant growth-regulator, typified by 2,4-dichlorophenoxyacetic acid.

In the present study the morphological changes which plants undergo following

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treatment with O-isopropyl N-phenyl carbamate are more fully described with a view to aiding investigators concerned with such problems as those raised in studies of growth-regulating compounds for vegetational control.

Avena sativa L. was chosen for the major portion of this work, since, in general, its responses are fairly typical of those of the grasses, and because the writer has used this species in studying cyto-histological responses to this carbamate. Many other species have been tested, and those which are responsive to this compound will be considered.

Experimentation

RESPONSE OF GERMINATING SEEDS TO SOIL APPLICATIONS

Germinating cereal plants have been observed to be very susceptible to soil applications of O-isopropyl N-phenyl carbamate (1). In view of the ease and rapidity with which plants can be tested by this method, over fifty species have been so tested at the germination stage. Immediately following planting, the compound, in 100 ml. aqueous solution, was applied to the soil in $\frac{1}{2}$ -gallon pots at a rate of 10 mg./1.7 kg. soil. The soil was a mixture consisting of two parts very fine sandy loam and one part peat by volume. Observations were made of the gross responses of the plants as manifested in emergence, morphological changes, and subsequent growth (table 1).

There were striking differential effects upon the emergence of various species. Some emerged from soil containing the carbamate without showing any obvious response; others failed to emerge. The effects were not limited to monocotyledons. Many dicotyledonous species were affected.

RESPONSES OF SOME MONOCOTYLEDONOUS SPECIES.—The gross responses of

germinating cereals and grasses to this substance were very similar. Five days after application no emergence was noted in pots containing susceptible species (table 1). The more resistant gramineous species, such as corn, sudan grass, rice, sorghum, and millet, were retarded in emerging and had enlarged coleoptilar regions. All corn plants failed to develop further, whereas 30–50% of the total number of sorghum, sudan grass, millet, and rice plants grew slightly. The latter plants were greatly retarded, and their newly emerged leaf blades were about 10–20% the width of those of untreated plants. The root systems of the treated plants were greatly stunted; no adventitious roots had developed within 10 days after treatment. The more susceptible species (oats, barley, rye, wheat, timothy, redtop, orchard grass, and rye grass) did not emerge above the soil surface. Macroscopic examination of the latter abnormal seedlings revealed that the shoot had burst through the seed coat and that the coleoptilar region had become greatly enlarged. The roots of the susceptible cereals were $\frac{1}{4}$ – $\frac{1}{2}$ inch long and were stubby and bulbous (fig. 1), whereas in the case of rye grass and timothy no roots were formed. Up to 3 weeks after treatment neither root nor shoot growth had been initiated from seed of redtop or orchard grass.

RESPONSES OF SOME DICOTYLEDONOUS SPECIES.—Most of these species tested did not show obvious responses to application of this carbamate at the germination stage (table 1). All the cucurbits tested, however, responded in varying degrees. Cucumber and watermelon barely emerged from the soil, and their cotyledons failed to expand. Such seedlings had short, thick hypocotyls and poorly developed root systems (fig. 2A). Pumpkin, squash, and gourd were slightly de-

TABLE 1
EFFECT OF O-ISOPROPYL N-PHENYL CARBAMATE UPON GERMINATION OF DIFFERENT PLANTS
APPLICATIONS TO SOIL AT PLANTING TIME AT RATE OF 10 MG./1.7 KG. SOIL
IN 100 ML. AQUEOUS SOLUTION

Common name	Scientific name	Response*	Comments
Chenopodiaceae			
Garden beet.....	<i>Beta vulgaris</i> L.	x	
Swiss chard	<i>Beta vulgaris</i> var. <i>cicla</i> L.	x	
Compositae			
Chicory.....	<i>Cichorium intybus</i> L.	x	
Sunflower.....	<i>Helianthus annuus</i> L.	x	
Convolvulaceae			
Morning-glory.....	<i>Ipomoea grandiflora rubro-coerulea praecox</i>	xxx	Delayed in emergence; permanently stunted; dark-green color
Cruciferae			
Mustard.....	<i>Brassica juncea</i> Coss.	xx	Shortened hypocotyl
Rape.....	<i>Brassica napus</i> L.	x?	
Broccoli.....	<i>Brassica oleracea</i> var. <i>botrytis</i> L.	x	
Cabbage.....	<i>Brassica oleracea</i> var. <i>capitata</i> L.	x	
Turnip.....	<i>Brassica rapa</i> L.	x	
Radish.....	<i>Raphanus sativus</i> L.	x	
Cucurbitaceae			
Watermelon.....	<i>Citrullus vulgaris</i> Schrad.	xxx	Delayed in emergence; permanently inhibited at 2-leaf stage
Cucumber.....	<i>Cucumis sativus</i> L.	xxx	Delayed in emergence; permanently inhibited at 2-leaf stage
Squash.....	<i>Cucurbita maxima</i> Duchesne	xx	Slight delay in emergence; shortened hypocotyl
Pumpkin.....	<i>Cucurbita pepo</i> L.	xx	Slight delay in emergence; shortened hypocotyl
Gourd.....	<i>Lagenaria vulgaris</i> Ser.	xx	Some shortening of hypocotyl
Euphorbiaceae			
Castor bean.....	<i>Ricinus communis</i> L.	xx	Delayed germination, shortened hypocotyl

* x—No apparent effect; xx—Plants delayed in germination and stunted but some subsequently recovered; xxx—Plants emerged but developed no further; xxxx—No emergence.

TABLE 1—Continued

Common name	Scientific name	Response*	Comments
Gramineae			
Red top.....	<i>Agrostis alba</i> Auct.	xxxx	No seeds germinated
Abyssinian oat.....	<i>Avena abyssinica</i> Hochst.	xxxx	Enlarged coleoptile, bulbous roots
Red oat.....	<i>Avena byzantina</i> C. Koch.	xxxx	Enlarged coleoptile, bulbous roots
Wild oat.....	<i>Avena fatua</i> L.	xxxx	Enlarged coleoptile, bulbous roots
Oat.....	<i>Avena sativa</i> L.	xxxx	Enlarged coleoptile, bulbous roots
Animated oat.....	<i>Avena sterilis</i> L.	xxxx	Enlarged coleoptile, bulbous roots
Sand oat.....	<i>Avena strigosa</i> Schreb.	xxxx	Enlarged coleoptile, bulbous roots
Orchard grass.....	<i>Dactylis glomerata</i> L.	xxxx	No seeds germinated
Sorghum.....	<i>Holcus sorghum</i> L.	xx	Delayed emergence; some permanent stunting, others recovered; hairlike leaves
Sudan grass.....	<i>Holcus sudanensis</i> Bailey	xx	Delayed emergence; some permanent stunting, others recovered; hairlike leaves
Barley.....	<i>Hordeum vulgare</i> L.	xxxx	Enlarged coleoptile, bulbous roots
Rye grass.....	<i>Lolium multiflorum</i> Lam.	xxxx	No roots developed; bulbous coleoptiles
Rice.....	<i>Oryza sativa</i> L.	xx	Germination reduced; stunting
Timothy.....	<i>Phleum pratense</i> L.	xxxx	No root development; bulbous coleoptile
Rye.....	<i>Secale cereale</i> L.	xxxx	Enlarged coleoptile, bulbous roots
Millet.....	<i>Setaria italica</i> Beauv.	xx	Delayed emergence; some permanent stunting, others recovered; hairlike leaves
Wheat.....	<i>Triticum sativum</i> Lam.	xxxx	Enlarged coleoptile, bulbous roots
Corn.....	<i>Zea mays</i> L.	xxx	Reached plumule leaf stage; dark-green coloration
Leguminosae			
Peanut.....	<i>Arachis hypogaea</i> L.	x	
Lespedeza.....	<i>Lespedeza striata</i> Hook. & Arn.	x	
Alfalfa.....	<i>Medicago sativa</i> L.	x	
White sweet clover.....	<i>Melilotus alba</i> Desr.	x	
Bean.....	<i>Phaseolus vulgaris</i> L.	x	
Field pea.....	<i>Pisum arvense</i> L.	x	
Garden pea.....	<i>Pisum sativum</i> L.	x	
Soybean.....	<i>Soja max</i> Piper	x	
Alsike clover.....	<i>Trifolium hybridum</i> L.	x	
Ladino clover.....	<i>Trifolium repens latum</i>	x	
Broad bean.....	<i>Vicia faba</i> L.	x	
Hairy vetch.....	<i>Vicia villosa</i> Roth.	x	
Cowpea.....	<i>Vigna sinensis</i> Endl.	x	
Linaceae			
Flax.....	<i>Linum usitatissimum</i> L.	xxxx	Hypocotyl swollen and shortened

TABLE 1—Continued

Common name	Scientific name	Response ^a	Comments
Malvaceae			
Cotton.....	<i>Gossypium hirsutum</i> L.	x	
Polygonaceae			
Buckwheat.....	<i>Fagopyrum esculentum</i> Moench.	xxxx	Hypocotyl swollen and shortened
Solanaceae			
Pepper.....	<i>Capsicum frutescens</i> (L.) var. <i>grossum</i> Bailey	xxx	Reduction in stand; permanently inhibited at 2-leaf stage
Tomato.....	<i>Lycopersicon esculentum</i> Mill.	xxx	Delayed emergence; permanently inhibited at 2-leaf stage
Tobacco.....	<i>Nicotiana tabacum</i> L.	xxx	Reduction in stand; permanently inhibited at 2-leaf stage
Petunia.....	<i>Petunia violacea</i> Lindl.	xxx	Reduction in stand; permanently inhibited at 2-leaf stage
Eggplant.....	<i>Solanum melongena</i> (L.) var. <i>esculentum</i> Nees.	xx	
Umbelliferae			
Carrot.....	<i>Daucus carota</i> (L.) var. <i>sativa</i> DC.	x	

layed in emerging and also had short, thick hypocotyls. They appeared stunted up to 2 weeks after treatment, but subsequently they fully recovered.

Buckwheat and flax plants responded similarly to each other in that the seeds germinated and broke the surface of the soil, but the shoot failed to emerge. Examination of the sprouted seedlings showed that the hypocotyl was much shortened and thickened. The root system was stunted, and the root tips were swollen. The cotyledons partially unfolded beneath the soil surface but failed to develop further (fig. 2B, C).

Morning-glory plants were delayed in emergence, and the leaves appeared rugose and were inhibited in unfolding. These plants had swollen and shortened

hypocotyls and thick primary roots. The leaves were distinctly darker green in color 10 days after treatment. Although the cotyledons remained green for over 3 weeks, no further shoot growth was produced (fig. 2D).

Tobacco failed to emerge from treated soil. Tomato and pepper emerged, but 3 weeks after treatment they had not produced any epicotyledonary leaves. The tomato seedlings had a distinctly darker green coloration 7-10 days following application and very short hypocotyls.

RELATION OF EXPOSURE INTERVAL TO SURVIVAL OF OAT AND BARLEY PLANTS

An experiment was performed to determine the relationship between length

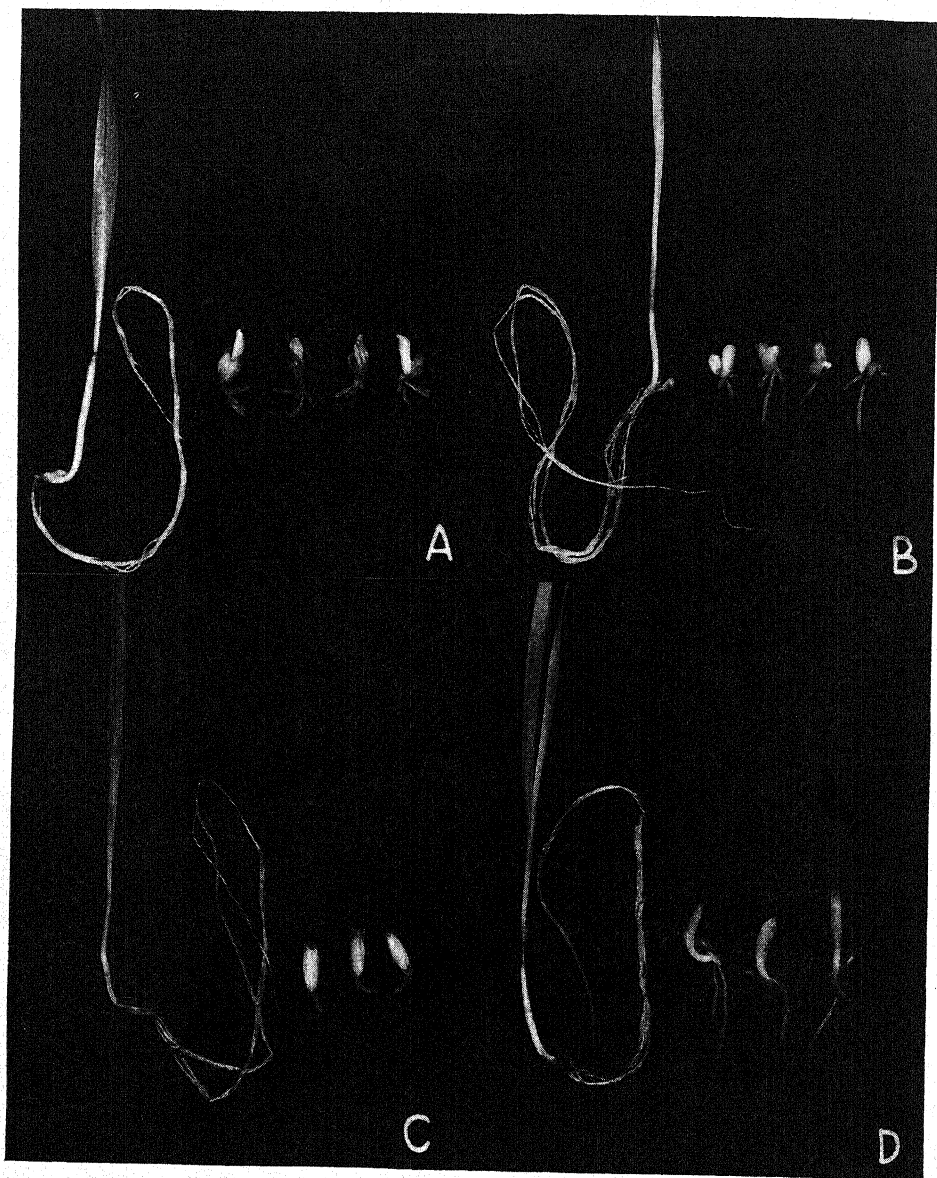


FIG. 1.—Response of germinating cereals to soil applications of O-isopropyl N-phenyl carbamate. Applications of 100 ml. aqueous solution were made at planting time at rate of 10 mg./1.7 kg. soil. Note enlarged coleoptiles and stubby, bulbous roots; control on left in each case. A, barley; B, wheat; C, oats; D, rye. (Photographed 7 days after treatment.)

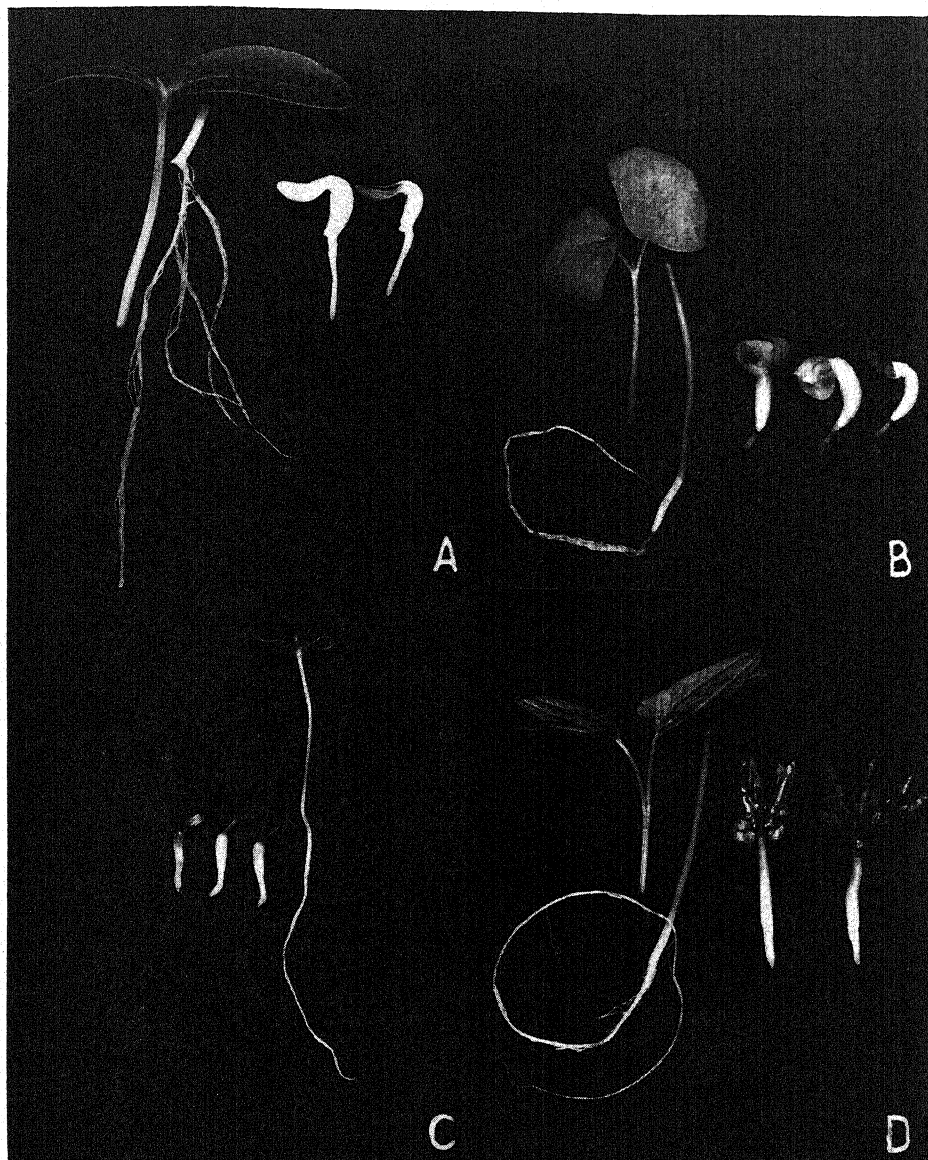


FIG. 2.—Response of certain germinating dicotyledonous plants to soil applications of O-isopropyl N-phenyl carbamate. Applications of 100 ml. aqueous solution were made at planting time at rate of 10 mg./1.7 kg. soil. Note short, thick hypocotyls and poorly developed, thick radicles. Shoot growth ceased at this stage. Control on left in each case except in C. A, cucumber; B, buckwheat; C, flax; D, morning-glory. (Photographed 7 days after treatment.)

of exposure to the carbamate and survival of the treated plants. Four-day-old oat and barley plants growing in soil in glazed pots were treated in April, 1947, by applying the carbamate, in 250 ml. of aqueous solution, to the soil at rates of 0, 1, 5, 10, and 50 mg./1.7 kg. soil. At various intervals ranging from 1 to 120 hours after application, twenty-five to thirty plants were removed from the variously treated soils. After all soil was carefully washed from the roots, they were transplanted to small paper drinking cups containing soil with no carbamate. Eight to 10 days later each surviving plant was transplanted to a glazed pot containing 1.7 kg. soil with no carbamate and was moved into the open, outside the greenhouse. Observations were made of the number of plants surviving, growth habit, number of tillers, and heads or panicles produced. Representative plants were photographed at intervals from the seedling stage to maturity.

Since cytological studies upon oat plants exposed to O-isopropyl N-phenyl carbamate show that abnormal nuclear and cell behavior occurs within a few hours following treatment (5), root and shoot material was fixed at various stages of the experiment to determine the relation of these early induced responses to the survival and subsequent development of oat and barley plants.

Three days after 4-day-old oat plants were exposed for 1 hour to 5, 10, or 50 mg. of this substance per pot, stunting was apparent, and the first foliage leaf had a dark blue-green coloration. Barley plants similarly treated showed the same responses but to a lesser degree. Four days after plants were exposed to 5, 10, or 50 mg. per pot, marked inhibition was observed in both cereals. The first foliage leaves were spatulate and dark green. The 1-mg. treatment resulted in slight

inhibition. After a 24-hour exposure to 5, 10, or 50 mg. of the carbamate per pot some bulbous swelling of the root tips was noticed; after 30 hours the coleoptile ruptured in certain cases, and the coleoptilar nodal region had a slight yellowish coloration.

With increasing intervals of exposure, the gross effects described above became marked, except in the pots treated with 1 mg. of the substance; many plants recovered from this latter treatment.

Many plants did not show swelling of the coleoptilar region when removed from treated soil, but such swelling subsequently developed after their transfer to soil with no carbamate. This indicated that a sufficient quantity of carbamate or stimulus produced by the compound was present in the tissues to result in gross swelling even after they were placed in untreated soil.

All plants retaining a green color were transplanted to glazed pots 12 days after treatment. Some of them had only a dark blue-green first foliage leaf protruding from the coleoptile, whereas others had put out additional leaves or, in some cases, tillers which had much narrower leaves than the controls (fig. 3). Some plants showing no shoot growth had developed new roots. This may have been the result of unequal exposure of the roots and shoots to the carbamate but more likely to a greater capacity of the plant to initiate new roots than new shoot growth.

Figure 5 shows the effect upon survival of treating oat plants with the carbamate for varying intervals. Barley responded similarly. Plants which showed gross responses at the time of transplanting were greatly delayed in growth and developed a rosette habit (fig. 44); many subsequently died. In the early stages of development a greater degree of tillering was

evident in the treated plants, but the untreated plants later produced about the same number of tillers. Many plants were delayed several weeks in maturity (fig. 4*B*). In view of certain similarities between the cytological effects induced by this substance and by colchicine, seed from the treated plants was collected, and plants grown from them will be examined cytologically to determine possible increase in chromosome numbers and whether morphological changes were induced.

Oats exposed to 50 mg. of the carbamate per pot for 1 hour showed less than 50% survival and only about 10% when exposed for 6 hours (fig. 5). All plants died after exposure to 10 mg. per pot for 30 hours, and only about 5% of the plants survived after being exposed to 5 mg. for 36-48 hours. Barley plants were more capable of surviving than oat



FIG. 3.—Oat plant 32 days after exposure for 12 hours to soil containing 5 mg. of O-isopropyl N-phenyl carbamate per 1.7 kg. soil.

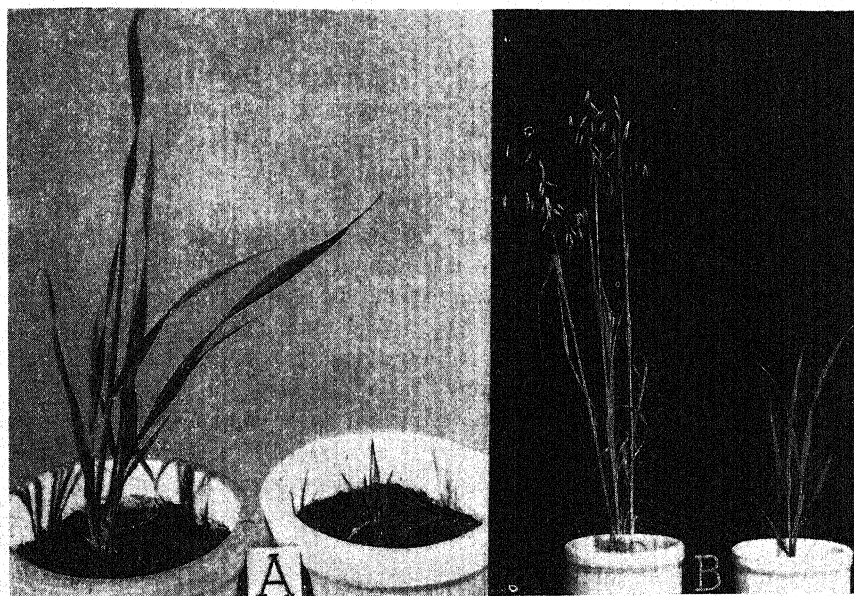


FIG. 4.—Response of oats to soil applications of O-isopropyl N-phenyl carbamate. *A*, 32 days after 4-day-old oat plant was exposed for 24 hours to soil containing 5 mg. carbamate per pot. Note stunted, rosette growth. *B*, 69 days after 4-day-old oat plant was exposed for 6 hours to soil containing 5 mg. per pot. Note great delay in heading. Controls on left in both photographs.

plants under similar conditions, except at the high rate of 50 mg. The 1-mg. rate was usually a sublethal amount, and consequently most of the plants recovered even after long exposure (fig. 5).

CYTOLOGICAL AND HISTOLOGICAL RESPONSES OF BARLEY AND OATS

A study of slides prepared from oat and barley material described in the preceding section showed that cytological responses occur in these species after 1-6

verse took place. Exposure of cereal plants to this carbamate in either soil or nutrient-solution cultures resulted in cessation of cell division in root and shoot meristems with accompanying increase in chromosome numbers and great enlargement and maturation of these cells. Young differentiating leaf cells and the cortical and stelar root cells in the region of elongation likewise increased in size and matured.

RESPONSES OF ROOT.—Cytological responses were evident in roots of 4-day-old oat and barley seedlings 1 hour after application of O-isopropyl N-phenyl carbamate to the soil in which the plants were growing. Roots from oat plants treated at a rate of 10 mg./1.7 kg. soil showed increased mitotic activity in the stelar cells in the region of elongation, characterized by a disproportionately larger number of metaphase figures and fewer of anaphase than occurred in roots of the control. Applications of 50 mg. per pot resulted in chromosome clumps in both oats and barley. There was a lack of congression of chromosomes at the metaphase plate; instead they were scattered throughout the cell. Anaphase figures were scarce in this material, and many of those which were present appeared abnormal in that chromosomes were separated into more than two groups, indicative of multipolar spindle action. These groups later formed micro-nuclei.

All the histogen cells of the root appeared to be slightly less responsive to treatment with the compound than the stelar cells basipetal to the histogen region. The increased activity of the stelar cells was perhaps related to the state of nuclear division at time of treatment or to a difference in the metabolic state of the histogen cells and those in process of elongation or expansion.

After 6 hours of exposure to 50 mg. of

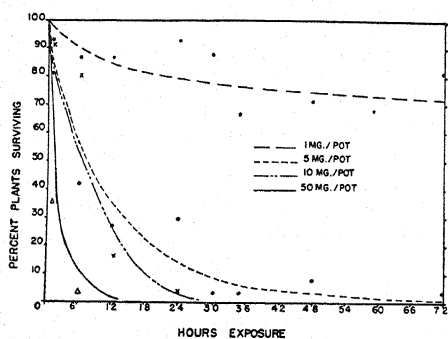


FIG. 5.—Effect of O-isopropyl N-phenyl carbamate upon survival of 4-day-old oat plants exposed to substance for varying intervals. Applications to soil at four rates in 250 ml. of water. Percentages based on number of plants reaching fruiting stage.

hours of exposure to O-isopropyl N-phenyl carbamate in soil. It appears pertinent to record certain of these responses which may be associated with survival of the plants and with certain morphological characteristics which developed after exposure to the compound for varying intervals.

In contrast to 2,4-dichlorophenoxyacetic acid and similar growth-regulating substances, O-isopropyl N-phenyl carbamate had a profound effect on meristematic cells of root and shoot. Instead of the rapid induction of cell division in such tissues as endodermis, pericycle, or rays, which results from applications of 2,4-dichlorophenoxyacetic acid, the con-

O-isopropyl N-phenyl carbamate per pot, oat roots showed abnormal anaphase figures and many metaphases. In treated barley roots there were three to four times as many metaphases as anaphases. Although untreated barley roots showed fewer anaphases than metaphases, the proportion was far less than that in the treated roots. In the abnormal anaphases the chromosomes were often separated into several clumps. Cytokinesis appeared to have largely ceased.

Thirty hours after treatment with the carbamate at a rate of 10 mg. per pot, the cortical and stelar cells of oat roots had many micro-nuclei. These cells were located a few cells basipetal to the histogen layer and extended into the region of elongation of the root. Such cells were frequently vacuolated and contained unevenly stained cytoplasm. Many blocked metaphases occurred, and the chromosomes in the stelar cells were markedly crowded and showed an obvious increase in number. Some cortical cells had giant nuclei, while others had micro-nuclei. Very few anaphase figures were present in the cortical and stelar cells which showed the numerous blocked metaphases. The cells in adventitious roots were twice the diameter of those in the control and contained larger nuclei.

After 72 hours essentially all root cells basipetal to the histogen region were highly vacuolate, and many cells contained several micro-nuclei (10-12 per cell, in some cases). The large mass of nuclear material in some cells was usually irregular in shape. Cells had become greatly enlarged.

RESPONSES OF SHOOT.—Cytological behavior of shoot cells was similar to that occurring in the root. Following exposure to 50 mg. of O-isopropyl N-phenyl carbamate per pot for 1 hour, the provascular cells in the leaves of 4-day-old oat and

barley seedlings showed a large number of metaphases with chromosomes scattered throughout the cell in a diakinesis-like manner. This did not occur in untreated plants. The chromosomes were contracted, and true anaphase separation did not occur as observed in untreated plants. The provascular region of the differentiating leaves showed the greatest activity, as characterized by the presence of numerous metaphase-like figures. The occurrence of so many of these figures was suggestive of an anesthetizing action of the carbamate when cells are past the prophase with consequent failure of the mitotic cycle to go to completion.

After 6 hours cells of the young leaves and of the apical meristem showed an accumulation of numerous blocked metaphases and a few abnormal or no anaphase figures. Only slightly fewer anaphases than metaphases occurred in untreated plants. There were some cells showing clumps of chromosomes of unequal size.

Thirty hours after 4-day-old oats were exposed to 10 mg. in soil, giant nuclei were present in certain cells of the apical meristem and leaves of the shoot. These cells were greatly enlarged and matured. The enlargement was approximately proportional to the increase in nuclear material.

RESPONSES OF ESTABLISHED CEREALS

APPLICATION IN NUTRIENT CULTURE.—

The germination stage appears to be the most susceptible for inducing responses with this carbamate. Studies have indicated that, with increasing age, cereal plants have a greater tolerance to the compound when applied in the soil. This may result in part from the fact that plants in later stages of development have deeper root systems; thus, the lower part of the root system may not be in

contact with the substance applied in the surface layer.

A study was made of the effects of this carbamate on well-established plants in which the entire root system was exposed. Oat plants were grown in coarse gravel supplied with Hoagland's nutrient solution, and the carbamate was introduced in the solution at concentrations of 0.1, 1.0, and 5.0 p.p.m. when the plants were 4 days old, 6 inches tall, and in early and late boot stages.

velopment was inhibited if the roots were exposed to the compound even at the time the panicles were emerging from the boot. Four-day-old seedlings exposed to concentrations of 1.0 or 5.0 p.p.m. often had ruptured coleoptiles a few millimeters acropetal to the coleoptilar node.

In the experiment reported above it was noted that, a few hours after roots of oats were exposed to concentrations of 1.0 p.p.m., shoot elongation ceased. In order to observe the effects of extremely

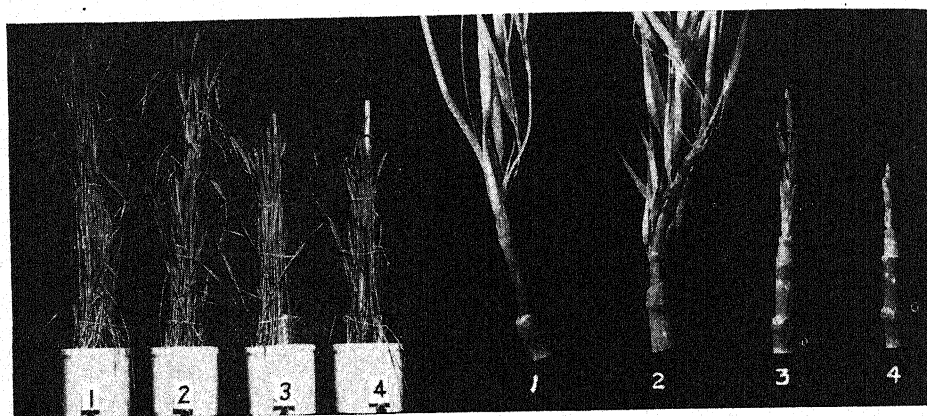


FIG. 6.—Effect of exposing oats to O-isopropyl N-phenyl carbamate in gravel nutrient culture for 11 days. Plants 35 days old when initially treated. On right are panicles excised from plants on left. 1, control; 2, 0.1 p.p.m.; 3, 1.0 p.p.m.; 4, 5.0 p.p.m. Note that 1.0 or 5.0 p.p.m. of the substance has caused cessation of shoot elongation.

Contrary to effects resulting from exposures in soil (2), plants at each of these stages of growth failed to develop further when exposed to 1 or 5 p.p.m. of the substance. No new roots were formed, and those already present developed bulbous tips with profuse root-hair growth. The leaves became dark blue-green; subsequently a brown discoloration developed at the tip which gradually progressed to the base of the blade. Failure of the growing point to develop further and of the young internodes to elongate resulted in markedly suppressed top growth and panicle development (fig. 6). Panicle de-

dilute concentrations of this substance on shoot elongation, eleven enamel trays covered with paraffin nets were set up and filled with Hoagland's nutrient solution containing 0, 0.1, 0.2, . . . , 1.0 p.p.m. of the carbamate. Thirty 4-day-old oat seedlings of uniform shoot length were transferred to each tray, and the roots immersed in the solution.

Seven days later the shoots were measured (cotyledonary node to tip of the longest leaf blade). Shoot elongation was inversely proportional to the concentration of the solution (fig. 7).

APPLICATION TO TOPS.—Earlier work

with this carbamate (2, 4) has indicated that it has relatively little effect upon cereals when applied only to the tops of seedling plants. Since the concentration of the carbamate in the nutrient solution which resulted in inhibition of the shoot was relatively low, it appeared that the amount of compound actually in the inhibited shoot was probably very small. It accordingly seemed that direct application of the compound to the shoot might result in pronounced responses if the growing-point were brought into contact with the substance. Consequently, plants were treated at the seedling stage and at the boot stage both by spraying and by dipping the tops into solution.

The aerial portions of some oat plants 15 inches tall (soil surface to tip of longest leaf) and of others in the "boot" stage of development were dipped twice into aqueous solutions³ containing 100, 1000, or 5000 p.p.m. of the carbamate. The plants were thoroughly wetted, since the formulation contained a wetting agent. Control plants were similarly dipped in water containing Santomerse D. All plants were allowed to dry with the pots in a horizontal position to prevent contamination of the soil. Oat plants at the same stages of development were sprayed six times over a period of 2 days with 50 ml. per sq. yd. of a 5000-p.p.m. aqueous solution. The soil was protected from the spray in one series with cotton batting. Responses were observed until the control plants matured.

No effects were induced when the tops of 15-inch plants were treated by either dipping or spraying, whereas plants in the boot stage were inhibited by both types of treatment. The response of

plants in the boot stage to these top applications were like those described in the nutrient-solution experiment for plants in the boot stage (fig. 8). Plants sprayed with the carbamate when 15 inches tall, with soil not protected from the spray, were killed by the treatment (fig. 9). The same spray treatment at the boot stage, however, was no more effective in inhibiting panicle emergence than the treatment in which the soil was protected

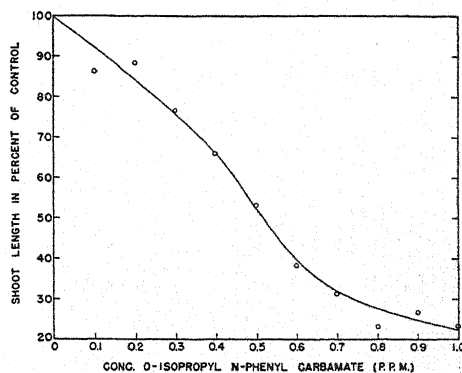


FIG. 7.—Effect of O-isopropyl N-phenyl carbamate upon shoot elongation of oats. Roots exposed continuously to substance in Hoagland's solution. Plants 4 days old when first exposed and measurements taken after 7-day exposure.

(fig. 9). The difference in response at the two ages may be explained on the basis of stage of development. At the early stage the internodes had not elongated, and the apical meristem of the shoot was well protected by many enveloping leaf sheaths. On the other hand, the internodes of a plant in the boot stage had fully elongated, and the growing-point was protected only by the sheath of the flag leaf and by perhaps one other leaf. Consequently, treatment of tops at this later stage caused inhibition of the embryonic tissues of the panicle either through absorption and translocation of the carbamate through the leaves or by direct external exposure of the meristem

³ Solutions made from a 20% experimental formulation of O-isopropyl N-phenyl carbamate prepared by John Powell & Co., New York City.

to the substance as a result of its penetration through the leaf sheaths or between their folds.

Certain field experiments have been carried out to observe the effect of this carbamate on wheat when applied at different stages of development. Owing to

tall and in the boot stage. Plots were 5×18 feet; areas 3×18 feet were treated. Cloth shields 5 feet in height were used to prevent drift of spray. Ap-

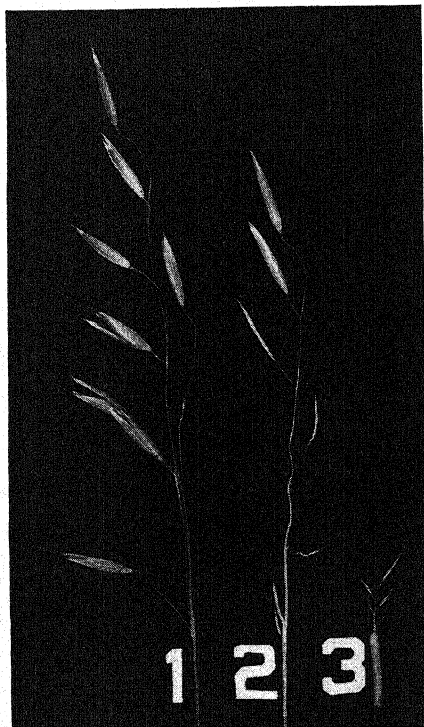


FIG. 8.—Effect of dipping tops of oat plants in aqueous formulations of O-isopropyl N-phenyl carbamate. Plants in boot stage of development when treated. 1, 1000 p.p.m. Santomerse D (control); 2, 1000 p.p.m.; 3, 5000 p.p.m. Twenty days after treatment.

the low solubility of this compound in water, it was applied in solution in 10 ml. of oil at rates of 0.5, 1.0 and 1.5 gm./sq. yd. The substance was first dissolved in tributylphosphate (1 gm./2 ml.) and then diluted to the appropriate concentration with no. 2 fuel oil (6). Applications were made in quadruplicate when the plants were 6 inches and 15 inches

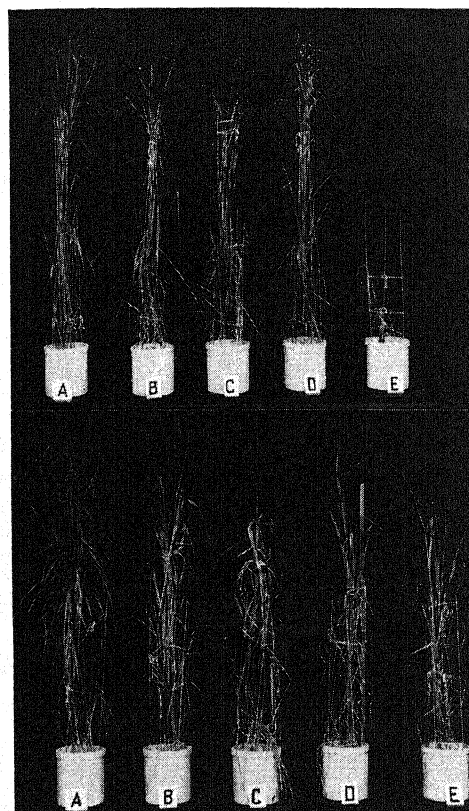


FIG. 9.—Effect of O-isopropyl N-phenyl carbamate upon oats. Plants treated by dipping or spraying the tops at 15-inch stage (*above*) and boot stage (*below*) with aqueous formulations of substance; 46 and 20 days after treatment, respectively. A, control; B, 1000-p.p.m. dip; C, 5000-p.p.m. dip; D, 5000-p.p.m. spray (soil covered); E, 5000-p.p.m. spray (soil not covered). Plants below are about equally inhibited by all treatments.

plications were made with a DeVilbiss type MBC spray gun fitted with Decorators MBC-231 spray head and a veiling cap which produced a medium-fine drop-let spray. Gross responses and effect on grain yield were recorded.

This study and other comprehensive

field studies, not reported here, are in agreement with the greenhouse studies, since applications of this compound in oil spray at late stages caused similar inhibition of cereal plants. Spray applications at earlier stages reduced the growth of cereals very effectively, but these effects appeared to be induced almost wholly as a result of soil contamination. Although burning of the plants by the tributylphosphate-oil formulation caused considerable reduction in grain yield at all stages, wheat plants sprayed at the 6-inch or boot stage yielded significantly less than those treated at the 15-inch stage (fig. 10).

IRRIGATION WATER TREATMENTS OF RICE.—Rice plants, 19 days old, were treated by applying the carbamate in irrigation water. They were much stunted, developed a dark-green coloration, and produced many tillers. Twenty-eight days after treatment such plants had not increased in height (fig. 11). Forty-two days after treatment the plants were considerably recovered, in that shoot growth, although somewhat stunted, had occurred and panicles had begun to emerge from the main culms and tillers. At this time the control plants were in the hard-dough stage. These effects were unlike those caused by sublethal applications of certain halogen-substituted phenoxyacetic acids since the latter usually cause no marked delay in maturity.

Forty-five-day-old rice (early boot stage) treated similarly also ceased to grow acropetally and became dark green. Fifty days after treatment, when grain was mature in the untreated plants, panicles in the treated plants had not emerged, although the plants were still green (fig. 11). Some new tillers were produced; it appeared, however, that plants treated at this later stage were less

capable of resuming apical growth or initiating new tillers than were plants treated at an earlier stage.

RESPONSES OF CERTAIN ESTABLISHED DICOTYLEDONS

Limited studies were made of the effects of this carbamate on several species. These were treated by applying the compound to the soil at relatively early stages of growth.

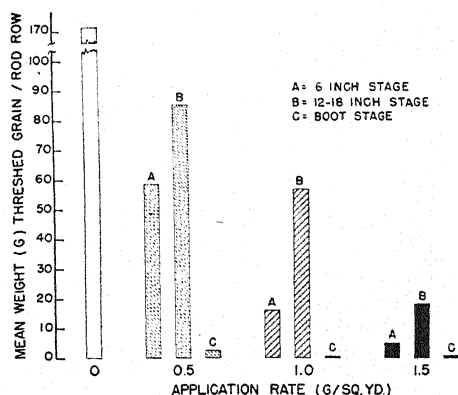


FIG. 10.—Effect of O-isopropyl N-phenyl carbamate upon yield of field-grown wheat. Compound applied at three rates in tributylphosphate-oil spray. *F* values for stages, rates, and stages \times rates are highly significant.

Fagopyrum esculentum was one of the first plants to be described as being affected by O-isopropyl N-phenyl carbamate (2). In order to test the effects of top application of this substance further, buckwheat plants were exposed to it by dipping the tops into solutions of the carbamate at 100, 1000, or 5000 p.p.m. The same formulation was used as previously described for oats. Plants were also treated by placing over the growing-point absorbent cotton pledgets which were dampened five times with concentrations of 200, 1000, or 2000 p.p.m. of the compound during a 64-hour period.

The responses induced as a result of

each type of treatment were the same. The internodes failed to elongate, and the youngest leaves ceased to expand and became thickened. The floral parts on the main stem were reduced in number (fig. 12). Axillary shoots subsequently developed. In some instances the shoots

which developed at the cotyledonary node were very short and bore two hundred or more flowers. Untreated plants showed no shoot development at the cotyledonary node, and shoots arising at the other nodes bore only 50 to 75 flowers each. Buckwheat treated at sub-

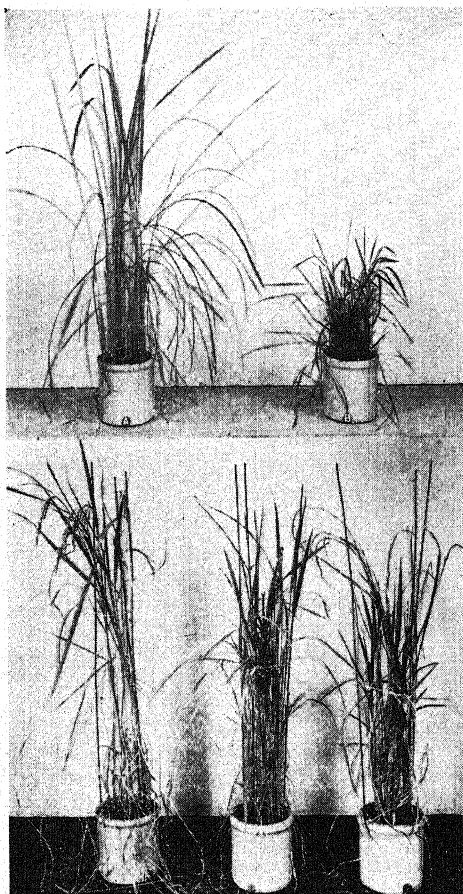


FIG. 11.—Effect of O-isopropyl N-phenyl carbamate upon rice when applied in irrigation water. *Above (right)*, 28 days after application, in 0.1 ml. fuel oil, of 25 mg. per pot. Plants 15 inches tall when treated. Note darker color and profuse tillering. *Below (center)*, 49 days later, same pot as on right above. Panicles are emerging. *Right (below)*, treated at same rate when 45 days old (36–40 inches). No panicles had emerged 50 days after treatment. Treated plants developed darker green color in all cases. Controls on left in both photographs.

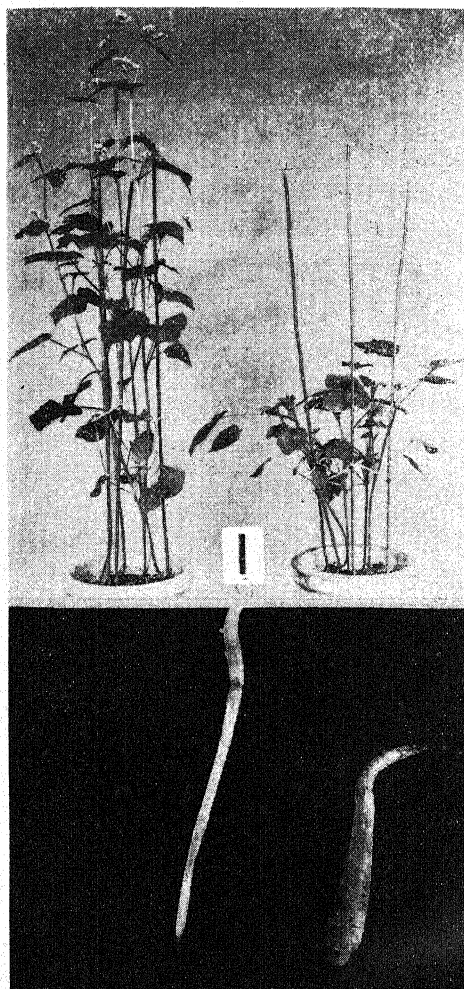


FIG. 12.—Effect of O-isopropyl N-phenyl carbamate upon buckwheat. *Above (right)*, plants treated by applying aqueous solution of carbamate at 200 p.p.m. to cotton pledgets placed over apical buds. *Below (right)*, root tip from buckwheat exposed to substance in soil. Controls on left in each case.

lethal levels by soil or top applications produced axillary shoots even though the primary stem apex was permanently inhibited. The root tips became bulbous following soil applications (fig. 12).

The similarity of response to the two types of treatment with O-isopropyl N-phenyl carbamate is evidence that the compound is most effective on young or meristematic tissues. These observations are in accord with results obtained by dipping or spraying oats at a late stage.

Plants of *Solanum tuberosum*, 2 inches tall, treated with O-isopropyl N-phenyl carbamate at rates ranging from 8 to 20 mg. per pot by applying the substance to the soil, developed a rosette growth (fig. 13). The leaves became thickened and brittle. The terminal growing-point of the central stem was permanently inhibited, but 17 days following treatment axillary shoots had begun to develop and 3 weeks later had reached a height equal to the control (fig. 13). Tubers harvested from potatoes grown in the greenhouse indicated that the yield was not markedly affected by applications such as those described above.

Plants of *Lycopersicon esculentum*, 8 inches tall, treated by applying the carbamate to the soil at rates of 3, 5, 10, 15, or 25 mg./1.7 kg. soil, showed responses 4 days after treatment. The petioles were curled downward, and the leaves were distinctly rugose. Plants later became prostrate, probably as a result of a stunted root system. Subsequently, the apex of the stem turned upward with the lower stem supported by the rim of the pot (fig. 14). Internodal elongation was retarded, and, in plants treated at a rate of 25 mg. per pot, a marginal necrosis of leaves developed 10 days following treatment. Virtually no further growth occurred in such plants. Twenty-five days after treatment at rates of 10 or 15 mg.

per pot, many axillary shoots were developed, while the terminal bud was inhibited (fig. 14). This response resembled the responses of buckwheat and Irish potatoes similarly treated.

Discussion

Recent work on plant growth-regulating substances has been largely centered on their use for herbicidal purposes and, in particular, on the development of herbicidal methods by which undesirable weeds might be removed from a crop. Al-

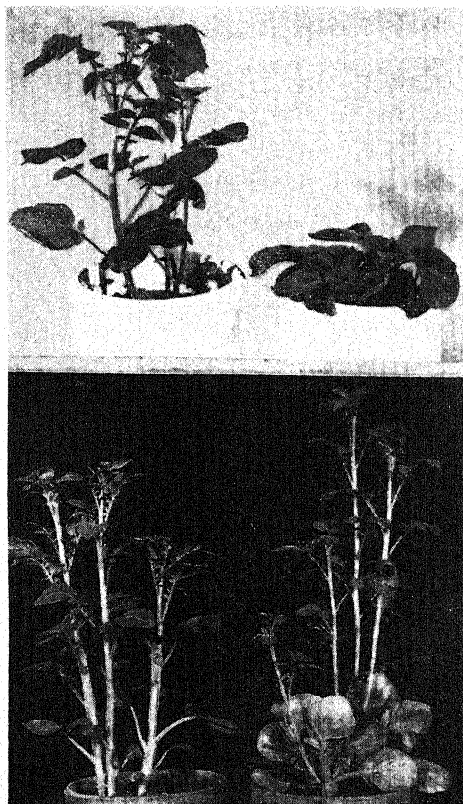


FIG. 13.—Response of Irish potatoes to soil applications of O-isopropyl N-phenyl carbamate. Above (right), rosette-like plant 17 days after application of 20 mg. to soil. Below (right), typical growth following recovery from treatment. Note that central stem is suppressed and that axillary shoots have been produced.

though O-isopropyl N-phenyl carbamate has inhibitory activity on the growth of certain plants, the responses induced by this substance are clearly of a different type from those induced by certain halogen-substituted phenoxyacetic acids,

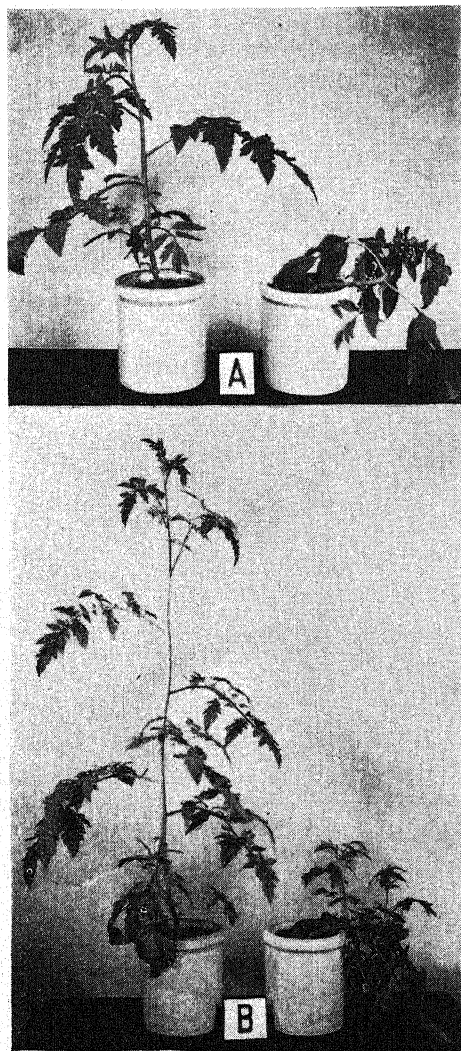


FIG. 14.—Response of tomatoes to soil application of O-isopropyl N-phenyl carbamate. *A* (right), plant 12 days after application of 10 mg. per pot. *B*, same plants as *A* 16 days later. Note production of axillary shoots and suppression of terminal bud in treated plant (cf. fig. 13). Controls on left.

of which 2,4-dichlorophenoxyacetic acid is the best known. At one time the latter was considered to be without effect on cereals and grasses, and, indeed, spray applications of 2,4-dichlorophenoxyacetic acid at low concentration may be made to established cereals and grasses without causing obvious injury. If applied, however, in greater concentration or to germinating seeds or seedlings, 2,4-dichlorophenoxyacetic acid appears to induce responses in virtually all species which have been tested. The compound O-isopropyl N-phenyl carbamate has been shown in this work to be more truly selective. Its activity, however, is not limited to monocotyledonous species as has been claimed in popular literature. O-isopropyl N-phenyl carbamate is distinctive in that at the germination stage all cereals and grasses tested showed responses to it, whereas dicotyledonous species varied in their response. Most dicotyledons germinated and grew in soil containing the carbamate without showing any obvious response, but under the same conditions certain other dicotyledons were severely inhibited. The compound did not induce epinasty in any species tested.

Responses can be induced in cereals by applications of the carbamate to the tops at certain stages of development. Effects can be induced also in some susceptible dicotyledonous species (such as buckwheat) through applications to the tops of established plants. The stage of development in cereals is a significant factor in the degree of their response to top applications. Cereals with fully elongated internodes—for example, the boot stage—are inhibited when the tops are dipped or sprayed with formulations of the carbamate. On the other hand, cereal plants with internodes not elongated do not show any obvious response to similar

treatments. The differential response in the two different growth stages is probably related to the position of the growing-point. For example, the young inflorescence of a cereal plant, with its elongated internodes, is protected from spray or dip applications of the carbamate by the sheath of the flag leaf and perhaps one other sheath; consequently, the inflorescence is inhibited by contact with the compound which can readily penetrate between the folds of the leaf sheaths or be absorbed and translocated through the leaves to the young tissues of the inflorescence. Prior to elongation of the internodes of cereals the stem apical meristem is protected from the spray by many enveloping leaf sheaths; no responses, therefore, are induced by applications of the carbamate to tops of such plants.

Although responses can be induced in cereals with fully elongated internodes by applications of O-isopropyl N-phenyl carbamate to their tops, other work with cereals shows that responses are most easily induced by applications of the carbamate to the soil at the germination and young seedling stages. With the formation of deep and extensive root systems, correspondingly higher rates of application of the carbamate to the soil are required in order to cause inhibition of cereals and susceptible dicotyledonous species.

As previously outlined, mitotic aberrations occur in the root and shoot of young oat and barley plants after an exposure to O-isopropyl N-phenyl carbamate in soil for a very few hours. Cytological studies on cereals treated with the compound show that the action of the carbamate is related primarily to an interrupted mitotic cycle and to failure of cytokinesis in both root and shoot. Depending on the interval or intensity of

exposure, abnormal growth or death of the cereal plants occurs as a result of the early induced cytological disturbances. Obviously, the mode of action of this carbamate is unlike that of the halogen-substituted phenoxyacetic acids.

In spite of the fact that O-isopropyl N-phenyl carbamate has not proved to be satisfactory as a herbicide for the control of weedy grasses, such as the deep-rooted perennials, and can improbably be developed for this purpose, nevertheless the specificity of action of this compound on certain species at the germination stage offers considerable encouragement. This carbamate might be applied to the soil with impunity at the germination stage to control susceptible obnoxious plants without causing concurrent injury to nonsusceptible crop species. The demonstrated differential response of various crop species to this compound suggests that in the development of new herbicidal methods there is a need for comprehensive screening of the effects of many growth-regulators on many different species.

Cereals with elongated internodes (boot stage) failed to produce seed and were stunted by applications of the carbamate to the tops alone. Correspondingly, in the use of this substance, and perhaps other similar compounds, for the control of established grassy weeds, applications to the tops would likely be most effective if applied at the jointing stage, preferably in a penetrating carrier such as a light oil. Use of some oils would not be advisable, however, for selectively controlling weeds in most dicotyledonous crops because of damage to the latter plants by the oil. Applications of compounds similar to this carbamate to tops only of grassy weeds before the internodes elongate would probably cause little systemic effect.

Growth-regulating substances have been used not only as herbicides but for inducing parthenocarpy and rooting of cuttings, for delaying fruit abscission, and for many other purposes. Little attention has been given by agronomists to their probable usefulness in controlling growth of agronomic plants. Certain responses induced by some of these compounds suggest that growth-regulators may be useful tools in attacking certain vegetational control problems. In pastures, for example, it is often desirable to keep plants in the succulent vegetative stage. If the plants reach the flowering stage, they become woody and unpalatable. Since, under some circumstances, O-isopropyl N-phenyl carbamate markedly delays maturity of rice and certain dicotyledonous plants, without killing them, growth-regulators of this general nature may have some usefulness in preventing fruiting of certain plants, flowering of which is controlled by specific photoperiods, or for delaying maturity in certain other species.

The carbamate causes mitotic aberrations and an increase in chromosome number in certain tissues of root and shoot of cereal plants; therefore, the substance may offer promise for experimentally inducing polyploidy or perhaps mutations in plants. Since chromosomes of oats and barley treated with the carbamate become contracted and the mitotic cycle is blocked at metaphase, the compound may find use in counting and studying the morphology of chromosomes in certain species.

Summary

1. Thirteen monocotyledonous species treated with O-isopropyl N-phenyl carbamate at the germination stage showed similar gross responses to the compound. These were characterized by lack of root

and shoot elongation, with concurrent swelling of these parts. The roots were stubby and bulbous, and the coleoptilar region was markedly swollen.

2. Young established cereals treated by soil application of the carbamate ceased to grow acropetally, and the leaves became dark green. In contrast to responses induced by applications of 2,4-dichlorophenoxyacetic acid, no epinasty occurred.

3. Of thirty-nine dicotyledonous species exposed to O-isopropyl N-phenyl carbamate at the germination stage, fifteen showed some responses to the compound. Plants of six of these species largely recovered from the treatment, whereas in nine they were permanently inhibited. All the latter species responded similarly in that the hypocotyl failed to elongate normally and became enlarged. The root system was much stunted, and in some species, such as buckwheat, flax, and morning-glory, only a radicle emerged. Most of the species responding to the carbamate developed to the two-leaf stage, but the cotyledons never fully expanded, and the stem apex failed to grow.

4. Applications of the carbamate to the tops of oat plants in the boot stage of development resulted in cessation of panicle growth, whereas similar applications at the seedling stage induced no responses. The relation of these responses to stage of plant development and certain implications regarding the use of other similar growth-regulators are discussed.

5. Marked abnormal cytological behavior occurred in the roots and shoots of oat and barley plants treated with O-isopropyl N-phenyl carbamate. This was characterized by an interrupted mitotic cycle, blocked metaphases, multinucleate cells, occurrence of giant nuclei,

and a highly increased chromosome number in certain cells of both root and shoot. Cell division ceased in the apical meristems of root and shoot. Great cell enlargement and maturation occurred in these cells and in those in the process of expansion.

6. The abnormal cytological behavior occurring in the root and shoot of oat and barley plants treated with the carbamate can be correlated with subsequent survival of the plants or with abnormal development. Short-time exposure studies revealed that, as the cytological effects became more pronounced, the number of plants surviving to maturity was markedly reduced.

7. Possible agronomic applications of growth-regulators similar to O-isopropyl N-phenyl carbamate for vegetational control are suggested, as well as the probable usefulness of this carbamate in genetic and cytological studies of cereals and certain grasses.

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INFLUENCE OF 2,4-DICHLOROPHENOXYACETIC ACID ON INITIATION AND DEVELOPMENT OF HYPOCOTYLEDONARY BUDS OF DECAPITATED FLAX¹

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 599

RICHARD M. KLEIN AND GEORGE K. K. LINK

Introduction

Literature bearing on the development of hypocotyledonary buds of flax after decapitation has been reviewed previously (2, 3, 5). The effects of indoleacetic acid on the different phases of this phenomenon have been investigated (6), but no investigation has been made of it using any of the synthetic growth-regulators. In view of known effects of 2,4-dichlorophenoxyacetic (2,4-D) on growth, it was thought that an investigation of the influence of this substance on the wound-reaction complex leading to the formation of new shoots might be of some interest and value.

Literature bearing on the mode, site, and time of initiation of such buds in flax has been reviewed and extended by LINK and EGGERS (5). The influence of 2,4-D in stimulating bud development has been noted by TAYLOR (8). He found that 0.04 p.p.m. in nutrient solution, with a presentation time of 9 days, stimulated axillary bud development from the cotyledonary node of cowpea and that the same concentration stimulated rooting from the lower hypocotyl of the kidney bean. Complete suppression of budding in both species was noted as a result of using a 0.6-p.p.m. concentration. VAN OVERBEEK (9) found that liquid applications containing 5 and 10 p.p.m. of 2,4-D, when placed on the apical buds on several varieties of pineapple,

induced a 100% response of flower-bud formation, while 1 p.p.m. induced no change. It is significant that similar concentrations of naphthaleneacetic acid were as effective as those of 2,4-D.

Material and methods

In all experiments a single lot of Bison variety of flax (1946 planting) was used. (Seed more than 2-3 years old does not give maximum germination, and the plants tend to be spindly.) The work was carried on from July through October, 1946, and from March through October, 1947, in the greenhouses of the University of Chicago and in a basement room under fluorescent lights. Details of the latter room are given later. Moderate temperature and high humidity appear to be optimal for seedling development, but neither could be maintained in the greenhouse. By trial it was found that 4- to 6-inch unglazed clay pots were more satisfactory containers for growing the plants than were the glazed earthenware pots originally used.

In preliminary studies in 1946, plants in soil showed a very high percentage of kill after decapitation. For this reason, all subsequent studies were made with no. 3 white quartz sand as a substrate, sifted after each experiment to remove roots and other debris.

Because plants in early experiments were lost after decapitation through fungal-induced damping off (*Fusarium* spp.), the seed in subsequent plantings were treated for at least 24 hours with a

¹ This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

"New Improved" Ceresan dust. The pots were watered with tap water until seedling emergence, and then with the complete nutrient of EGGERS (3) without adjustment of the pH. This was supplemented with soluble iron phosphate once a week in the proportion advocated by HELGESON *et al.* (4). No additional water was supplied.

Sprouting was usually complete within 3-5 days after sowing, and the seedlings were thinned to twenty-six to twenty-eight plants per pot. When the cotyledons were expanded and the epicotyl had begun to elongate (3-7 days after sprouting), the hypocotyl was cut at right angles to the axis just below the cotyledonary node. Twenty-five plants in each pot were so treated. One or more uninjured plants were left in each pot as a check on substrate and microclimatic conditions. These uninjured plants indicated an absence of gross environmental differences among the pots in individual experiments.

Mixtures of 2,4-D in lanolin or in Carbowax 1500, and in a range of concentrations, were applied to the decapitated stumps of the plants immediately after cutting. Two inocula were used: a large inoculum consisted of capping the cut surface of the hypocotyl with approximately 0.01 ml. of the paste; the small inoculum was about one-half as large. Sometimes it was necessary to remove the guttation fluid with filter paper before capping. The same number of decapitated untreated and carrier-treated plants were used as controls.

Examinations for detectable buds were made with a 2 \times hand lens at periods up to 18 days after application. This time limit was arbitrarily chosen, since one shoot usually attains dominance in that time (5), and the further development of additional buds is repressed.

Specimens for microscopical examination were fixed and stained by the method used by LINK and EGGERS (5).

Observations

EFFECT OF CARBOWAX.—One of the most striking results was the finding that the decapitated hypocotyl invariably died when Carbowax 1500 was applied to it either alone or as the carrier of 2,4-D, irrespective of the concentration of the acid. Neither this carrier alone nor the acid in it was toxic when applied to intact seedlings.

The sequence of necrobiotic change in the cut treated hypocotyl was as follows: Within 24 hours after application of either Carbowax alone or as a carrier for 2,4-D, there was a noticeable decrease in the turgidity of the cut treated hypocotyl as compared with either uncut Carbowax-treated or cut untreated plants. The upper 1-2 mm. were completely whitened by the end of this period. Freehand or frozen sections showed that, although the tissues outside the pith appeared essentially normal, there was plasmolysis and shrinking of the pith tissue. The whitened areas showed no chlorophyll, but the plastid "ghosts" were seen in normal position. Forty-eight and 72 hours after application of Carbowax there was complete collapse of all tissues, with whitening almost to the soil level; the dried, whitened hypocotyls could not be sectioned by either freehand or paraffin methods. When Carbowax 1500 was placed alone on the uninjured hypocotyl, epicotyl, or cotyledons, the plant developed normally.

In a critical study of the toxicity of carriers, WITHROW and HOWLETT (11) found that Carbowax was very toxic to tomato leaf and flower tissue. BEAL (1) compared Carbowax and lanolin as carriers of phenoxy compounds and stated

that the effectiveness of the carrier depended on the growth-regulator employed. He noted, however, that with few exceptions there were heightened responses when Carbowax was the carrier. Because of the toxicity of this carrier, particularly in plants that may have an altered disposition (i.e., injured plants) to chemical influence, some of the effects attributed to growth-regulators in other studies may have resulted from the carrier. Thus the type of carrier employed, in combination with the physiological state of the organism, undoubtedly is an important factor in the response. In any case a great deal of careful work is needed to determine both the mode of entrance of such substances as Carbowax and their effects on plant metabolism.

EFFECT OF 2,4-D.—In experiment I, single large applications of nineteen concentrations of 2,4-D in lanolin, ranging from 0.001 to 1000 p.p.m., were made to decapitated flax hypocotyls immediately after cutting. Replicates of a hundred plants were used, and equal numbers of decapitated untreated and lanolin-treated plants were employed as controls.

Within 24–48 hours after application the four highest concentrations of the acid (125, 250, 500, and 1000 p.p.m.) had induced chlorosis or blanching at the cut end, extending 1 cm. from the cut in the 1000-p.p.m. lot to less than 0.5 cm. in the 125-p.p.m. group.² There was no attendant loss of turgidity within 48 hours, but within 13 days after application the 1000-p.p.m. lot had lost turgidity and appeared to be undergoing necrobiotic change. Downward curvature and twisting of the hypocotyls occurred on the second day after application in the plants treated with 75 to 1000 p.p.m., in degrees of severity roughly proportional to the

concentration of the acid. This twisting lasted until the ninth to thirteenth day after application. At that time recovery was complete in all lots except those treated with the two highest concentrations.

In experiment I hypocotyledonary buds were evident in both control lots on the sixth day after application. By the ninth day, pots treated with all except the five highest concentrations of 2,4-D had budded plants, but the lot treated with the highest concentration (1000 p.p.m.) showed no buds even on the twenty-first day. The number of buds increased at a fairly regular rate for any particular concentration. A summary of the survival and bud counts on the eighteenth day is given in table 1.

To determine the accuracy of these findings, experiment II was performed. The small inoculum of lanolin paste (0.005 ml./plant) was used since the large inoculum, used in experiment I, was difficult to standardize. Although no marked differences in survival were noted between experiments I and II, and the gross effects were similar, it was evident that the plants in experiment II were less sensitive to 2,4-D, for bud counts were greater than in experiment I (table 1).

Because it was found in experiment II that a concentration of 1000 p.p.m. did not completely suppress budding—in contrast to the results in experiment I—concentrations of 2000 and 4000 p.p.m. as small inocula were used in addition in experiment III. In general, results were not sufficiently different from those of the previous experiments to warrant discussion. Concentrations of 2000 and 4000 p.p.m. were fatal within 3 days after application.

Since the difference in the amount of lanolin paste applied per plant and dif-

² Similar chlorotic changes were noted with the use of high concentrations of indoleacetic acid (6).

ferences in environment might have been factors accounting for the dissimilarity in results of experiments I, II, and III, large and small inocula were used on simultaneous plantings in experiment IV. As a further check, a similar planting was

cut hypocotyls was 450 foot-candles. Following the lead of ROBINSON (7), who found that 13-15 hours of light per day permitted normal development of flax, the plants were exposed to a photoperiod of 14 hours. In no instance, however, was

TABLE 1
COUNTS ON EIGHTEENTH DAY AFTER APPLICATION OF 2,4-D IN LANOLIN TO
DECAPITATED FLAX. REPLICATES OF 100 PLANTS

2,4-D (P.P.M.)	NUMBER OF PLANTS						AVERAGE NUMBER BUDS PER PLANT WITH BUDS		
	Alive			With buds					
	Experiment			Experiment			Experiment		
	I	II	III	I	II	III	I	II	III
4000.....			0			0			0
2000.....			0			0			0
1000.....	87	82	67	0	10*	12*	0	1.4	1.9
500.....	95	99	82	1	25	18	0	1.5	1.6
250.....	99	100	96	0	82	60	0	1.9	2.4
125.....	100	100		17	91		1.2	1.8	
75.....	100	100		25	95		1.6	2.1	
50.....	100	100		44	97		2.5	2.1	
25.....	100	100		57	91		1.6	2.2	
10.....	100	99		50	91		2.3	2.1	
5.....	100	100		58	96		2.5	1.9	
1.....	100	99		51	90		2.3	2.3	
0.500.....	100	98		59	88		2.0	1.8	
0.250.....	100	90		75	82		3.1	2.1	
0.125.....	100	100	99	74	91	99	2.5	2.0	4.4
0.075.....	100	100		76	88		2.8	2.1	
0.050.....	100	99		84	93		2.8	2.0	
0.025.....	100	100		60	96		3.1	2.5	
0.010.....	100	100		83	95		2.4	2.5	
0.005.....	99	96		79	91		2.7	2.5	
0.001.....	99	86		82	83		3.6	2.8	
Lanolin only....	99	99	99	87	95	97	3.3	3.0	3.6
Cut only.....	98	100	98	87	94	98	4.8	4.2	4.4

* Buds in lower portion of hypocotyl only.

grown in the greenhouse until the time of application and then transferred to a basement room. The temperature in this room was stable at $23 \pm 3^\circ \text{C.}$, and the relative humidity was 60-70% during the 18-day test period. Illumination was provided by nine 36-inch white fluorescent tubes suspended in three reflectors about 15 inches above the substrate level. Light intensity at the level of the

growth in the basement room as good as in the greenhouse. The uncut plants in the basement were about two-thirds as tall as those in the greenhouse, had a tendency to topple, and their leaves were smaller. In the basement the leaves and stems became progressively lighter until, at the end of the experiment, they were a light yellowish-green.

The results of experiment IV are sum-

marized in table 2. As evidenced by the bud counts of plants grown under controlled conditions of light and temperature, the differences between the effects of large and small inocula are not sufficient for size of inocula to be considered as an important factor in the differences noted between the results of experiments I and II. Plants under lights were not so vigorous as those in the greenhouse, as

periments III and IV (August, 1947) when the greenhouse temperatures reached 40°-42° C., just below the melting-point of lanolin. Although the small inoculum merely capped the hypocotyl and did not run, the large inoculum ran freely over one-half of the stump.

Although it has been assumed (10) that the concentration of a growth substance so applied, rather than the quan-

TABLE 2
COUNTS ON EIGHTEENTH DAY AFTER APPLICATION OF LARGE AND SMALL INOCULA OF 2,4-D IN LANOLIN TO DECAPITATED FLAX, UNDER ARTIFICIAL LIGHT AND IN GREENHOUSE. REPLICATES OF 50 PLANTS

2,4-D (P.P.M.)	ARTIFICIAL LIGHT						GREENHOUSE					
	Large inocula			Small inocula			Large inocula			Small inocula		
	Alive	Plants with buds	Total no buds	Alive	Plants with buds	Total no. buds	Alive	Plants with buds	Total no. buds	Alive	Plants with buds	Total no. buds
4000.....	0	0	0	0	0	0	0	0	0	0	0	0
2000.....	0	0	0	0	0	0	0	0	0	0	0	0
1000.....	4	0	0	16	0	0	6	1*	3	28	6*	10
500.....	13	1	1	42	3	4	25	8	18	32	6	9
250.....	21	0	0	46	10	14	26	8	13	48	33	89
0.125.....	43	39	100	49	48	126	44	44	173	49	49	202
Lanolin only.....	38	32	70	46	35	65	34	34	140	49	49	174
Cut only.....				48	47	125				50	50	202

* Buds in lower portion of hypocotyl only.

evidenced by bud counts in the untreated and lanolin-treated decapitated controls. The differences between these two latter series have been shown to be statistically insignificant (6).

In the greenhouse trials, however, the possibility still existed that the differences in the responses of plants treated with small and large inocula might be significant. This necessitated further study in order to determine the relative importance of the size of the inoculum.

The lanolin paste had a tendency to run down the hypocotyl in hot weather. This was particularly marked during ex-

tity, will partially determine the degree of response, we may proceed on the hypothesis that the area of plant surface available for entrance of the substance (designated as "presentation area") is a factor governing the degree of response. It would then appear that running of the lanolin paste increased the presentation area and was the factor responsible in experiment IV for the greater inhibition of budding noted in the greenhouse in plants treated with the large inoculum, rather than initial application of a large inoculum. Experiments designed to test this hypothesis are now being conducted.

Tentatively, it is assumed that the differences noted among the experiments here reported, relative to survival, bud initiation, and development in any particular concentration range, resulted from differences in the physiological states of the plants in the different experiments as influenced by external and internal environmental factors.

Longitudinal serial sections were made to determine, in the totally grossly inhibited material in experiment IV, whether bud primordium initiation was inhibited or whether primordium development was prevented. Since LINK and EGGERS (5) had reported that nondecapitated hypocotyls bear bud primordia in the lower one-half to two-thirds of their length, randomized hypocotyls were fixed at the start of the experiment and sectioned to determine the longitudinal extent of initial primordia. With this information, surviving plants treated with 1000 p.p.m. of 2,4-D in lanolin were selected from both the greenhouse and the basement lots and sectioned to determine whether primordia other than those initially present had been formed.

In the pretreatment seedling stage, buds were present only in the lower half of the unwounded hypocotyl, confirming the findings of LINK and EGGERS (5). Examinations of the treated material showed that there was no development of bud primordia initially present in the lower half of hypocotyls treated with either large or small inocula. Likewise, no bud primordia were found in the upper half of treated hypocotyls. Since all buds and shoots observed in the material treated with 1000 p.p.m. were found in the lower portion of the hypocotyls (table 1), it can be concluded that high concentrations of 2,4-D prevent the initiation of buds and repress development of any such buds initially present. This

repression is usually complete, but occasionally a bud primordium develops into a shoot.

Discussion

A comparison of these experimental results with those of LINK and EGGERS (6) shows that the gross and microscopic effects of 2,4-D and indoleacetic acid on flax are very similar. With neither substance was there any noticeable stimulation of bud formation, and suppression of bud initiation and/or development followed the same pattern. Even microscopic findings with regard to inhibition of bud initiation and development are transposable for the two substances. Inverse relations between the concentration of the growth substance and the number of buds produced were found for both compounds, as were such effects as blanching and necrobiosis of plants treated with the higher concentrations. 2,4-D was effective, however, in lower concentrations than indoleacetic acid. Whether there is any corollary between the effects noted in these studies and the mode of action of these very different compounds is beyond the scope of this investigation. The work of VAN OVERBEEK (9) would indicate that in pineapple, also, there is this type of gross similarity of action between substituted phenoxy compounds (2,4-D and some of its salts) and compounds (naphtheleneacetic acid and some of its salts) more closely approaching those, such as indoleacetic acid, naturally found in plants.

LINK and EGGERS (6) indicated that the metabolic and nutritive status of a plant is a factor in determining the effectiveness of indoleacetic acid in affecting bud formation and development. The initial physiological state is equally important with respect to the response

to 2,4-D. Because of variation in physiological states, the data obtained from any one experiment are not directly comparable with those of another, and each experiment must be evaluated individually.

The variation in bud production in any concentration range, pot, or even from plant to plant, reduces the effectiveness of a statistical study, such as that used by LINK and EGGERS (6), in determining the relative importance of such findings as (a) time after treatment of the appearance of the first bud, (b) number of plants with buds, and (c) number of buds per plant. The gradation from complete bud inhibition, and/or death of the plant, to complete freedom from inhibitory and suppressing effects is so subtle that it, too, could not be critically evaluated under the experimental conditions available.

Summary

1. 2,4-Dichlorophenoxyacetic acid in lanolin paste was applied to the stumps of decapitated flax. Its effects on development of hypocotyledonary buds was investigated.

2. There was an inverse relation between the concentration of the acid in the lanolin paste applied to the wound and the number of buds formed. Concentrations above 75 p.p.m. were noticeably toxic and inhibitory, while those below 0.5 p.p.m. and down to 0.001 p.p.m. induced slight, if any, inhibition. There was no demonstrable stimulation of bud

formation with any of the concentrations used.

3. In plants in which 2,4-D induced complete inhibition of grossly visible buds, the initiation of bud primordia in the upper portion of the hypocotyl was also completely inhibited. Although buds present in the lower portion of the hypocotyl at the time of application were usually inhibited, occasionally one or more of them did develop, indicating suppression rather than complete inhibition.

4. The gross and microscopic findings pertaining to these phenomena are similar for both 2,4-D and indoleacetic acid, although the concentration of 2,4-D necessary to induce any particular syndrome was less than that required of indoleacetic acid.

5. The physiological state of the treated plants is an important factor in determining response (as measured by survival and bud counts) to any particular concentration of 2,4-D.

6. It is postulated that the surface area available for entrance of a growth-regulating substance (designated as "presentation area") is a factor in determining the degree of response to that substance.

7. Carbowax 1500 was invariably fatal to the decapitated hypocotyl, inducing whitening and dessication of the tissues; it was not grossly harmful to unwounded flax organs.

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EFFECTS OF SEVERAL PLANT GROWTH-REGULATORS ON FRUIT SET, YIELD, AND BLOSSOM-END ROT OF SIX TOMATO VARIETIES GROWN UNDER HIGH TEMPERATURES¹

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Introduction

The problem of flower abscission is one which may arise when tomatoes are grown in areas where the night temperatures are high (6, 7, 8). Abscission occurs, in our experience, when the mean minimum night temperature does not fall below 78° F. and the minimum day temperature is 83° F., rising to 90° F. or above. The plants make excellent vegetative growth under such conditions but set few fruits. Those which do form usually fail to enlarge properly. The numbers of fruits which set and the extent to which they enlarge vary considerably with the variety. In fact, much can be done toward the production of marketable fruits under such conditions by the choice of proper varieties.

The present work was done at Curaçao, a tropical island in the Dutch West Indies. Here the problem of flower ab-

scission of tomatoes was encountered during the hot seasons. Several plant growth-regulators were tested to determine whether they could be of use in solving this problem. This was also thought to be an interesting study because of conflicting results which had been obtained in their use in the field and under greenhouse conditions.

In a preliminary experiment using two tomato varieties, Pearson and Pritchard, and five growth-regulators—indolebutyric acid, beta-naphthoxyacetic acid, parachlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), and alpha (ortho-chlorophenoxy) propionic acid²—it was found that some of the treated plants produced over twice the yield obtained from the untreated controls. Response to indolebutyric acid was least, and, although the plants treated with it yielded more than the controls, it was not used in the later work, as space was limited. Since the two varieties gave different results, a number of varieties were grown in later tests.

¹ This work was done in the summer of 1946 while the authors were employed by the Curacaosche Petroleum Industrie Maatschappij (Dutch Shell Oil Co.) at Curaçao, Netherlands West Indies. Thanks are due the management of C.P.I.M., Director A. TROOST in particular, for permission to publish these results.

² This chemical was furnished through the courtesy of Plant Products Co., Eatondale Avenue, Blue Point, N.Y.

Material and methods

The responses of six varieties of tomato to four growth-regulators were studied with respect to amount of early yield, fruit size and quality, and occurrence of blossom-end rot. Three varieties were of the indeterminate type of growth: Indiana Baltimore, Michigan State Forcing, and Pan America; the other three were determinate: Pearson, Pritchard, and Victor. Seeds were planted on May 20, 1946, in flats filled with sand, and seedlings were transplanted, when about 5 inches high, into the experimental beds on June 20. Eight plants of each variety were used in each of five treatments, including the controls. The indeterminate plants were staked and pruned to one stem; the determinate ones were neither staked nor pruned. All were grown in full sun.

A gravel-culture technique was used. The beds, each 8×3 feet in area and 7 inches deep, were flooded automatically four times daily with a nutrient solution which had been developed to give good results under tropical conditions. The water used in making the nutrient solution contained 300-400 p.p.m. of chloride, but as the plants used water, the chloride concentration increased to 1000 p.p.m. Previous experience indicated that the use of chloride concentrations of the latter magnitude tended to result in small fruits and greater incidence of blossom-end rot. This partially explains the high values for these items shown in the data for the controls.

The four growth-regulators were made up in aqueous solution in the following concentrations:

Para-chlorophenoxyacetic acid	75 mg./l.
2,4-Dichlorophenoxyacetic acid	10 mg./l.
Beta-naphthoxyacetic acid	100 mg./l.
Alpha (ortho-chlorophenoxy) propionic acid	100 mg./l.

In preparing these solutions, the compounds were first dissolved in 5 ml. of denatured 95% ethyl alcohol; then water was added to make a liter. The concentrations used were chosen after consulting pertinent literature (1, 2, 3, 5, 9, 10).

A DeVilbiss atomizer, no. 261, was used to apply the spray to the flowers. The beds were examined every second day, and the flowers were sprayed once when fully opened.

Results

Data on yield and blossom-end rot were recorded. Harvest data were based on the yield from August 28, 1946, through September 25, 1946, a period of 28 days. The results are summarized in table 1.

Almost all the fruits produced after applications of the chemicals were of marketable quality. Some leaf modification was brought about by 2,4-D, para-chlorophenoxyacetic acid, and beta-naphthoxyacetic acid. However, even with leaf modification, the early fruit yield was apparently not impaired.

Measurements of the rate of enlargement of fruit which developed from blossoms sprayed with the growth-regulators were made. The data showed that the rate of enlargement, particularly in the early stages of fruit development, was much faster in treated fruits than in untreated controls.

The various treatments brought about the favorable effects generally of increased fruit size and increased yield. Para-chlorophenoxyacetic acid and beta-naphthoxyacetic acid, however, induced particularly outstanding results. With most varieties the former was more effective, but with others the latter was better. 2,4-D and alpha (ortho-chlorophenoxy) propionic acid induced only slightly better results in treated plants

TABLE 1

HARVEST AND BLOSSOM-END ROT DATA

TOMATO VARIETY	BETA-NAPHTHOXYACETIC ACID (100 P.P.M.)			ALPHA (ORTHO-CHLORO- PHENOXY) PROPIONIC ACID (100 P.P.M.)			PARA-CHLOROPHENOXY- ACETIC ACID (75 P.P.M.)			2,4-DICHLOROPHENOXY- ACETIC ACID (10 P.P.M.)			ALL TREATMENTS			CONTROLS		
	Average yield per plant (gm.)	Average fruit size (gm.)	% blossom-end rot	Average yield per plant (gm.)	Average fruit size (gm.)	% blossom-end rot	Average yield per plant (gm.)	Average fruit size (gm.)	% blossom-end rot	Average yield per plant (gm.)	Average fruit size (gm.)	% blossom-end rot	Average yield per plant (gm.)	Average fruit size (gm.)	% blossom-end rot	Average yield per plant (gm.)	Average fruit size (gm.)	% blossom-end rot
Indiana Baltimore	364	37	54	290	39	58	758	61	38	471	47	43	471	47	48	266	31	71
Michigan State Forcing . .	284	41	20	176	36	17	403	61	36	279	50	49	285	48	33	137	48	67
Pan America	65	35	72	181	36	31	204	71	69	83	51	68	133	47	60	46	31	72
Average of indeterminates	236	38	50	217	37	45	457	62	45	278	48	49	207	47	47	151	34	71
Pearson	683	81	27	204	48	42	944	116	32	635	77	24	616	85	31	520	64	48
Pritchard	383	50	48	209	40	34	471	55	55	353	50	52	376	48	40	233	45	33
Victor	651	58	68	504	50	59	607	50	59	457	62	49	578	54	61	427	56	44
Average of determinates	569	63	55	356	46	50	677	71	52	483	64	43	521	61	51	302	57	43
Average of all varieties	402	53	53	287	42	48	567	67	49	380	57	45	409	55	49	274	48	58

than were obtained in untreated controls.

There were widespread differences among the varieties in response to the treatments. In general, the size of the determinate fruits was not increased so much as that of the indeterminates in response to the growth-regulators. The increase in size of treated fruit from indeterminate plants was so marked as to result in marketable fruits in many cases as against fruit too small to market from the control plants.

In general, the indeterminate varieties responded more to treatment than the determinate ones with respect to total yield, although in each type the total yield of treated plants was greater than in the controls. Among the indeterminates, increases in yield of as much as three times that of the controls were induced in certain cases by para-chlorophenoxyacetic acid.

There was no consistent over-all correlation between the various treatments and the occurrence of blossom-end rot. With some varieties (e.g., Indiana Baltimore) the treatments lessened the incidence of the disease. Yet with Victor there was just as definite a trend in the opposite direction. In general, however, the incidence of blossom-end rot seemed to be reduced by the application of growth-regulators among the indeterminate varieties and increased among the determinate varieties. There is one prominent exception to this statement: Pearson, a determinate type, had its incidence of the disease greatly lessened by the treatments.

Discussion

The effects of high constant temperature noted here correspond well with the results of WENT's studies (6, 7, 8) on tomato growth under controlled environmental conditions. He found that

fruit production in the tomato required a night temperature below 25° C. The authors independently came to the same conclusion from field studies. Night temperature seems to be a limiting factor in commercial tomato-fruit production. A temperature either too high or too low prevents fruit set and development.

The data indicate that there is some causal factor in the occurrence of blossom-end rot other than an excess or deficient water supply; the latter is usually first mentioned as its cause. LYON *et al.* (4) mentioned other factors which have been correlated with the incidence of this disease. In the work reported here many such factors were kept as constant as was practical, particularly the watering, mineral nutrition, osmotic concentration of the nutrient solution, and the insecticidal and fungicidal spray program. The data, however, show a very striking correlation between the incidence of blossom-end rot in particular varieties and the application of certain growth-regulators. That there are great differences among tomato varieties in their susceptibility to blossom-end rot is well known. Perhaps the varietal differences with relation to this phenomenon are correlated with hormonal mechanisms.

It should be remembered that the beds were sprayed every second day. This was too often. The work was done in a commercial fashion, no attempt being made to shield the remainder of the plant from the spray. Thus, there were leaf modifications brought about by certain treatments. In preliminary experiments in which the flowers were sprayed only twice a week, no leaf modification was noted, even when 2,4-D was used. In these preliminary experiments all the tomato fruits were cut open and examined for unfilled cavities and uneven ripening. A

few had cavities, but very few had green spots in the ripe fruit. This was also true in the main experiment, so that after several random samplings all subsequent fruit harvested was marketed. It is interesting that practically no malformed fruit developed when the growth-regulators were applied in a spray.

Under these conditions, which were unfavorable for the setting of fruit on tomato plants, the use of growth-regulators did bring about an increase in size and yield. Whether growth-regulators would induce such results under field conditions favorable for fruit set has not been finally determined. It would seem, however, that the use of certain chemicals to bring about the setting of fruit in the field might be commercially practical under climatic conditions similar to those described here. There are also localities in the United States where the first flower cluster often abscises owing to cold, cloudy weather; the use of growth-regulators might change this condition. This would be particularly desirable in obtaining a good set on the first and second flower clusters for early yield of tomatoes grown on truck farms in such areas. Since growth-regulators induce an increase in tomato fruit set under conditions of high temperature which are unfavorable for normal fruit production, it would seem quite likely that they could do the same thing under conditions of low temperature which are unfavorable for fruit set.

Summary

1. Under conditions of high night temperature many tomato flowers normally failed to set fruit. When flowers were sprayed with certain growth-regulators—para-chlorophenoxyacetic acid, beta-naphthoxyacetic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), and alpha (or-

tho-chlorophenoxy) propionic acid—a higher total yield and larger fruit size were obtained than were produced by controls. Results in certain cases were very striking.

2. There was great varietal variation in response to the treatments with respect to fruit size, total yield, and incidence of blossom-end rot. In general, the indeterminate tomatoes seemed to be more responsive to the treatments than the determinates, both with respect to increases in fruit size as well as in total yields.

3. Considering all varieties together, no consistent correlation was found between application of growth-regulators and incidence of blossom-end rot. Considering individual varieties, however, there was a marked correlation, enough to suggest the possibility that hormonal mechanisms may be involved with this phenomenon. Growth-regulators affected the incidence of blossom-end rot both positively and negatively, depending on the tomato variety and the chemical used.

4. Para-chlorophenoxyacetic acid brought about, in general, the best fruit set and subsequent fruit development, with beta-naphthoxyacetic acid second. With several varieties, however, the latter was the more effective. The third most effective was 2,4-D, this being better than alpha (ortho-chlorophenoxy) propionic acid; the plants treated with the latter compound yielded slightly more than the controls.

5. Of the tomato varieties studied, whether the plants were treated or not, the determinate type was superior to the indeterminate for fruit production during the tropical hot season.

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INFLUENCE OF TOXIC CONCENTRATIONS OF MICRO-NUTRIENT ELEMENTS IN THE NUTRIENT MEDIUM ON VITAMIN CONTENT OF TURNIPS AND TOMATOES¹

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Introduction

During recent years extensive experiments have been conducted at this laboratory to measure the influence of mineral nutrition on the vitamin content of various plants. HAMNER *et al.* (2, 8, 12) have shown that large variations in relative concentrations of calcium, potassium, magnesium, nitrate nitrogen, sulfate, and phosphate in the nutrient medium do not result in any marked changes in the ascorbic acid or carotene content of either turnip greens or tomatoes, although the treatments ranged from deficient to toxic concentrations of the

macro-nutrient elements and were associated with marked differences in the growth of both plants. Although LYON *et al.* (22, 23, 24) found iron-deficient tomatoes to contain 26.1 ± 0.55 mg. of ascorbic acid per 100 gm. fresh weight of fruit as compared with a value of 20.0 ± 0.46 for controls, severe deficiencies of manganese, zinc, copper, boron, or iron supply were not, in general, associated with variations in ascorbic acid, riboflavin, or provitamin A content. In these and other experiments (11, 14) the influence of mineral nutrition was considerably less than that of certain other environmental factors (particularly one or more aspects of climate) in determining vitamin content.

It is the purpose of this paper to evaluate the influence of relatively high con-

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centrations of micro-nutrient elements in the nutrient medium on the vitamin content of turnip greens and tomatoes. The growth and development of turnips and tomatoes in sand culture were measured, and thiamine, niacin, riboflavin, and ascorbic acid contents were determined.

Material and methods

The basal nutrient solution used in these experiments was that shown by BERNSTEIN *et al.* (2) to be optimal for growth of turnip greens and by HAMNER *et al.* (12) to be near-optimal for growth of tomatoes. It had the following composition in terms of milli-equivalents of macro-nutrient elements per liter: calcium, 12.0; potassium, 4.5; magnesium, 9.0; nitrate, 17.5; phosphate, 2.8; and sulfate, 5.7. It also contained the following concentrations of micro-nutrient elements in terms of parts per million: B as H_3BO_3 , 0.5; Mn as $MnCl_2$, 0.5; Zn as $ZnSO_4$, 0.05; Cu as $CuSO_4$, 0.02; Fe as $FeC_6H_5O_7$, 5.0; and Mo as $(NH_4)_2MoO_4$, 0.05.

Several preliminary experiments were conducted in the fall of 1942 and the spring of 1943 to determine the maximum concentration of each micro-nutrient element that could be used without completely inhibiting growth. Although the results of preliminary investigations are not reported in detail, the following concentrations (in p.p.m.) of the various micro-nutrient elements were indicated as approximately maximum for our purposes for both plants: Mn, 100; Zn, 100; B, 60; Mo, 100; Cu, 50. Iron treatments were exceptional in that no toxicity symptoms were evidenced by either plant when supplied with 150 p.p.m. of iron in the nutrient medium. Precipitation of iron occurred in the nutrient when iron concentrations in excess of 150 p.p.m. were used. Thirty treatments in-

volving five different concentrations of each element were then used in the more extensive investigations on both plants.

The design of the experiment was that of a randomized block (9). Each replication consisted of two adjacent crocks, and each treatment was randomized within a block by the use of TIPPETT's randomization tables (34). Thus, the mean of ten plants is used as an estimation of the results produced by a given treatment.

In the first experiment, seed of the Shogoin variety of turnips were planted in the greenhouse on May 15, 1943, in flats containing pure quartz sand. A complete nutrient solution (12) was used during germination and the seedling stage. On June 4, when the plants were 20 days old and approximately 3 inches tall, uniform seedlings were transplanted outdoors into 2-gallon glazed crocks containing pure quartz sand, one seedling per crock. They were watered-in with distilled water and immediately supplied with their respective nutrient treatments. The crocks were alternately flushed with distilled water and supplied with their respective nutrient treatments on successive days.

Beginning on June 30, one complete block (one replicate of each of thirty treatments) was harvested per day for ease of accomplishing vitamin analyses. Thus, replicate differences are confounded with time of harvest. Data for the number of leaves, total length of leaves, and total fresh weight of tops were recorded for each plant. The longest fully expanded leaf from each plant was used for analyses of ascorbic acid and riboflavin. The midrib was removed, and one half of the leaf was immediately extracted for ascorbic acid analysis, the remaining half being extracted in the fresh condition for riboflavin analysis. The second

longest fully expanded leaf was dried at 65° C. for 4 days in a forced-draft oven, the dry weight recorded, and samples taken for niacin, thiamine, and selected mineral analyses. The root systems were washed as free from sand as possible, dried, and separated from sand particles by pulverizing the dried material with adhering sand and separating these components by a current of air.

In the tomato experiment, seed of an inbred strain of the Bonny Best variety was planted in flats in the greenhouse on May 15, 1943. The plants were supplied with basal nutrient solution during germination and the seedling stage. On June 12, when they were approximately 3 inches tall, uniform seedlings were transplanted into 2-gallon glazed crocks containing pure quartz sand, one seedling per crock. They were watered-in with distilled water and immediately supplied with their respective nutrient treatments. At this time all plants were placed outdoors and were trained upright. All axillary growth was pruned off twice weekly. The fruits were harvested on August 26 and 27, all copper treatments, for instance, being harvested on one day. Thus, in this experiment, replicate differences are not confounded with time of harvest.

The first four fruits to mature on each plant were analyzed for ascorbic acid content. In general, the first fruits from the various plants, regardless of treatment, tended to mature at the same time as did also successive fruits. Thus, by grouping results of ascorbic acid analyses on the first fruits per plant and comparing them with all fourth fruits, essentially another measure of environmental influence—time of maturation—is available in the experiment.

The fungus-growth method (13) was used for all thiamine analyses with de-

terminations made in sextuplicate. Riboflavin was determined by SNELL and STRONG's microbiological method (33) with duplicate determinations per sample. The chemical method was used in determining ascorbic acid with a modification described by MORELL (26). The aliquot, however, was titrated to an end point with standardized dye. Niacin was determined by the method of KREHL *et al.* (19) with duplicate determinations per sample.

Mineral determinations were made by the following methods: zinc, COWLING and MILLER (5); iron, SAYWELL and CUNNINGHAM (31); copper, COULSON (4), with modifications suggested by DRABKIN (6); molybdenum, MARMOY (25), with modifications suggested by ROGERS (28); boron, the 1,1'-dianthrimide method of ELLIS *et al.* (7); and manganese according to the Official method (1). The analyses were made on unwashed leaf material dried at 65° C. and ground with mortar and pestle.

Results

Analyses of variance were computed for each individual character (32). The results for both turnips (table 1) and tomatoes (table 2) show statistically significant differences between treatments to be inherent in the data. Although environment as measured by replication also produced significant differences in such characters as dry-weight accumulation in the turnip experiment, replication differences were measured and not included in intertreatment comparisons. Thus, valid differences which can be directly ascribed to treatments exist in both experiments.

It is clear from tables 1 and 2 that iron treatments used in these experiments were not associated with significant differences in any of the characters ex-

aminated; however, there was a highly significant inverse relationship between the concentrations of copper, boron, manganese, zinc, or molybdenum supplied in the nutrient medium and the growth of turnips or tomatoes (figs. 1, 2, 3). Furthermore, in the turnip experiment, as the concentration of these micro-nutrients was increased in the nutrient medium, the leaves were smaller and fewer in number. Also the percentage of tops or

leaves in the whole plant decreased. Similarly, fruitfulness in tomatoes was severely impaired with respect to both number of fruit and leaflets per plant and average size of fruit and leaflets in treatments receiving more than the normal quantity of these elements.

Dried plant samples were analyzed for selected micro-nutrient elements, and it is apparent (table 3) that statistically significant differences between treat-

TABLE 1
RESULTS OF ANALYSES OF VARIANCE FOR GROWTH CHARACTERISTICS OF TURNIP PLANTS

CHARACTERISTIC	F VALUES FROM ANALYSIS OF VARIANCE											
	Between treatments ^a of						Between replications ^a of					
	B	Cu	Fe	Mn	Mo	Zn	B	Cu	Fe	Mn	Mo	Zn
Fresh weight of tops.....	4.78*	87.45*	2.09	7.74*	13.29*	23.65*	0.62	1.76	0.38	1.48	3.47	1.58
Dry weight of tops.....	4.62*	56.35*	2.06	2.17	9.42*	37.51*	1.53	4.09*	4.25*	1.02	7.14*	1.53
Fresh weight of roots.....	9.68*	8.62*	2.17	4.84*	13.60*	28.62*	1.81	1.60	4.93*	5.59*	2.34	1.15
Dry weight of roots.....	17.10*	15.93*	2.43	4.76*	10.02*	37.90*	2.40	4.46*	3.31	4.49*	2.53	1.37
Total fresh weight of plant	7.52*	80.15*	1.54	7.20*	17.87*	33.67*	1.22	3.57	2.24	3.76	4.00*	1.50
Total dry weight of plant	7.51*	56.10*	2.69	8.12*	15.65*	49.65*	1.82	5.73*	5.41*	4.66*	8.48*	1.89
Tops as percentage of whole plant.....	2.98	5.12*	1.79	2.97	2.40	2.66	0.99	0.87	0.28	2.14	0.57	0.61
Percentage dry matter in tops.....	1.00	1.45	0.75	4.93*	5.31*	0.40	5.47*	2.78	1.76	14.03*	4.03*	0.95
Number of leaves.....	2.09	17.89*	1.11	1.15	8.50*	18.97*	0.92	2.17	1.34	1.12	2.14	1.29
Leaf measurements.....	3.27	35.34*	2.82	2.16	10.84*	32.00*	1.34	4.25*	0.81	1.28	1.21	0.35

^a Four degrees of freedom are available for this statistic; forty degrees of freedom are available for error variance.

* Highly significant; when $F=3.83$, $P=0.01$.

TABLE 2
RESULTS OF ANALYSES OF VARIANCE FOR CHARACTERISTICS OF GROWTH AND FRUITFULNESS OF TOMATO PLANTS

CHARACTERISTIC	F VALUES FROM ANALYSIS OF VARIANCE											
	Between treatments ^a of						Between replications ^a of					
	B	Cu	Fe	Mn	Mo	Zn	B	Cu	Fe	Mn	Mo	Zn
<i>Vegetative growth:</i>												
Height of vine.....	14.16*	49.50*	0.04	7.00*	73.48*	78.70*	1.01	2.25	0.33	0.03	0.92	1.30
Fresh weight of vine.....	73.80*	76.70*	0.94	8.24*	21.22*	85.59*	1.08	0.84	1.36	0.31	0.86	1.19
Dry weight of vine.....	234.24*	83.47*	0.34	11.46*	21.73*	38.36*	6.94*	1.69	0.62	0.60	0.16	0.31
Percentage dry matter.....	9.38*	4.63*	0.92	3.64	18.97*	3.47	0.72	0.96	0.67	0.62	1.84	0.43
Dry weight of roots.....	104.41*	35.48*	2.72	30.98*	136.44*	26.70*	2.82	1.02	1.48	0.40	1.41	2.40
<i>Fruitfulness:</i>												
Total weight of ripe fruit.....	32.85*	46.90*	0.06	60.33*	228.49*	61.74*	0.56	0.44	0.03	0.80	0.10	1.05
Number of ripe fruit.....	5.10*	15.89*	1.26	26.50*	110.30*	21.10*	0.89	0.28	3.00	1.90	0.70	0.40
Average weight of ripe fruit.....	11.01*	278.29*	1.30	22.70*	46.80*	16.21*	0.52	2.17	2.76	2.74	1.55	1.56
Total number of immature fruit.....	24.20*	16.40*	1.81	28.50*	50.60*	19.20*	0.00	1.20	1.96	1.40	3.90*	0.04
Total weight of immature fruit.....	6.50*	27.98*	1.26	59.90*	105.40*	44.22*	0.01	0.72	0.72	16.20*	1.00	0.69

^a Four degrees of freedom are available for this statistic; forty degrees of freedom are available for error variance.

* Highly significant; when $F=3.83$, $P=0.01$.

ments are inherent in the data. Although differences in environment between experimental replicates were associated with significant differences in chemical composition in three cases, the magnitude of chemical differences associated

Results of the chemical analyses are presented in figure 4. When the concentration of any micro-nutrient element was increased in the nutrient medium, both the tomato and the turnip plants accumulated it in their vegetative parts.

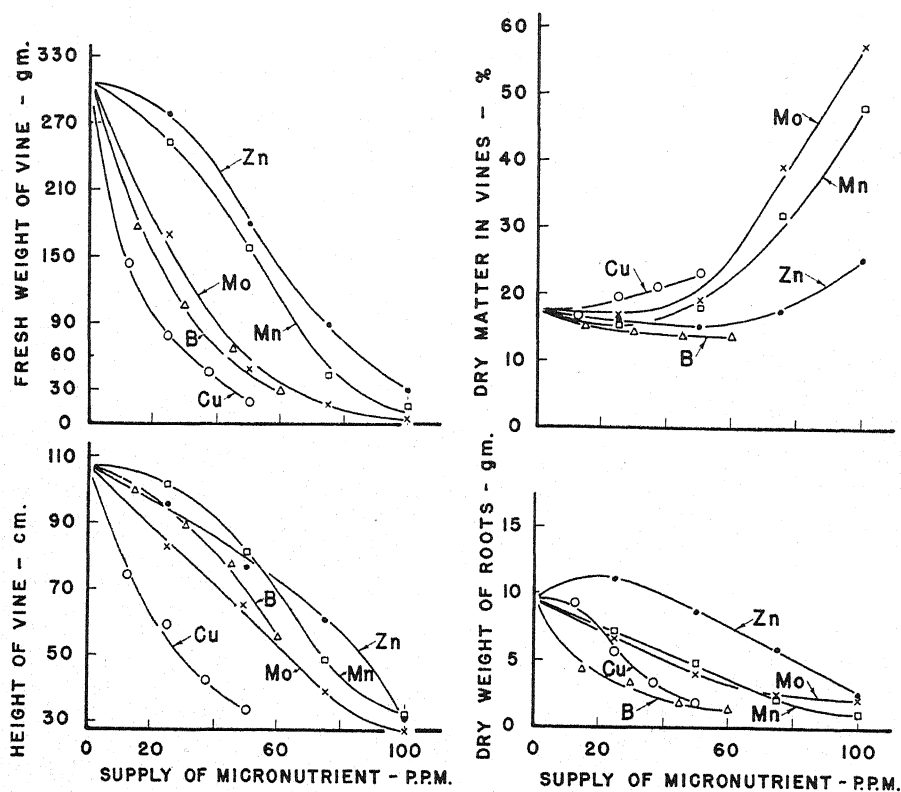


FIG. 1.—Effect of supply, in toxic concentrations, of micro-nutrient elements on growth of tomato plants.

with different environments was not great. Although plants supplied with relatively high concentrations of iron in the nutrient medium showed no external toxicity symptoms and were comparable with control plants with respect to characters of growth and fruitfulness, the highly significant *F* values between iron treatments denote significant differences in iron content of vegetative plant parts.

It is interesting that the concentrations of some of the elements were increased a thousand fold or more in the plant tissues, although the accumulation of iron was considerably less. There seems to be, also, no consistent relationship between the concentration of micro-nutrients supplied and the concentration in plant tissues. For instance, a thirty-fold increase in the concentration of iron supplied to turnips was associated with less

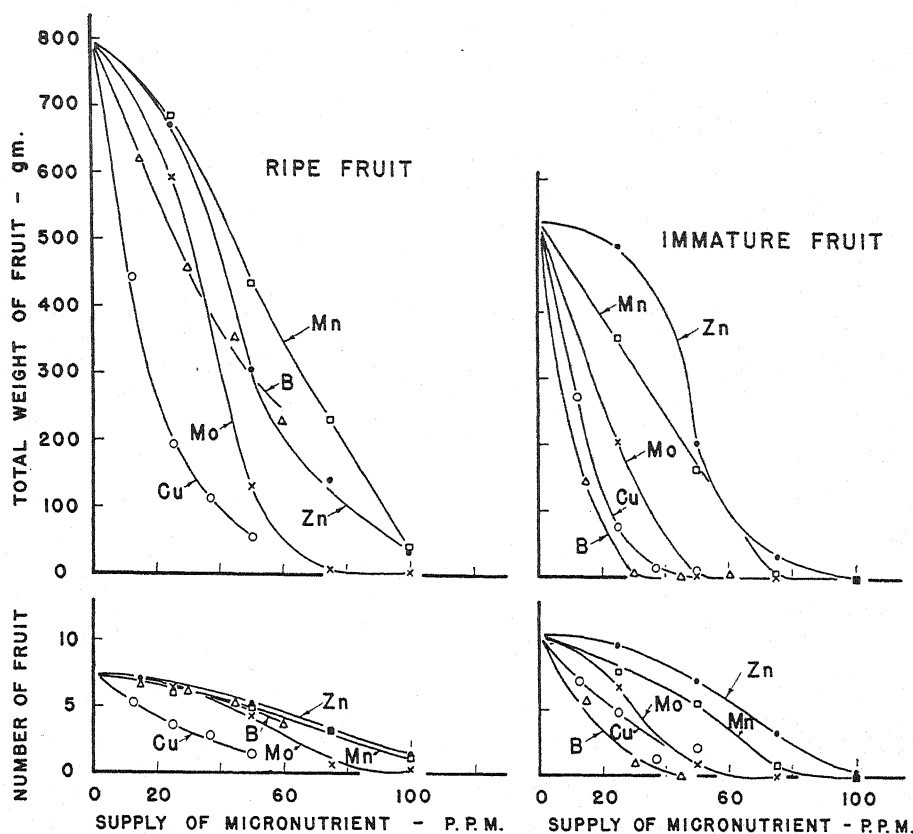


FIG. 2.—Effect of supply, in toxic concentrations, of micro-nutrient elements on yield of tomato fruit

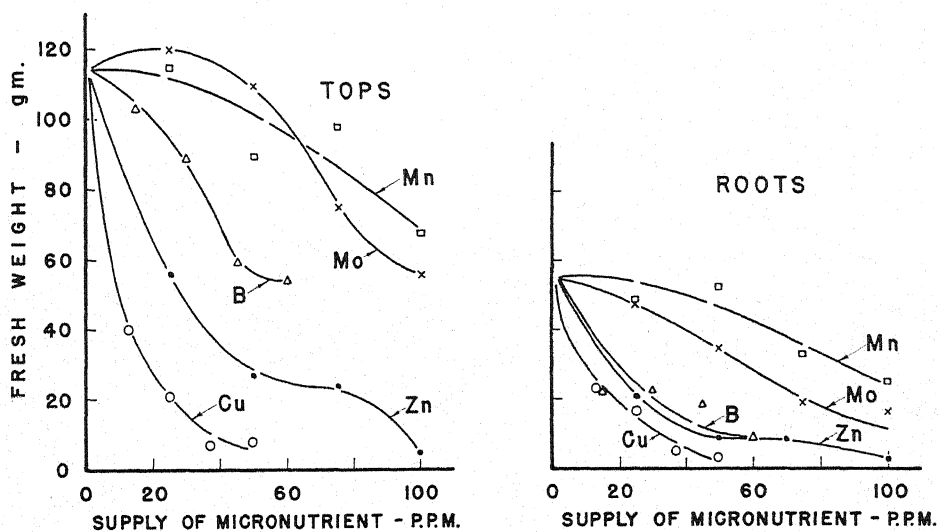


FIG. 3.—Effect of supply, in toxic concentrations, of micro-nutrient elements on growth of turnips

than a twofold increase in the iron concentration in the leaves. A fivefold increase in manganese supply to turnips resulted, however, in a fifteen-fold increase in the manganese concentration in the leaf. Perhaps the greatest accumulation rate was evidenced by a comparison of tomato plants supplied with 0.05 and 25.05 p.p.m. of molybdenum. In this

instance, more than a thousand-fold increase in molybdenum concentration in leaflets occurred. The data also show that, at the higher levels of supply, the rate of accumulation within the plant decreases as the level of supply is increased.

The data for thiamine, niacin, riboflavin, and ascorbic acid contents of

TABLE 3
RESULTS OF ANALYSES OF VARIANCE FOR ANALYTICAL DATA CONCERNED WITH CHEMICAL COMPOSITION OF TURNIPS AND TOMATOES. *F* VALUES ARE PRESENTED

MICRO-NUTRIENT ELEMENT	BETWEEN TREATMENTS		BETWEEN REPLICATIONS	
	Tomato leaflets	Turnip leaves	Tomato leaflets	Turnip leaves
Boron.....	122.22*	48.22*	3.47	3.16
Copper.....	14.02*	2.02
Iron.....	22.25*	16.01*	6.82*	0.42
Manganese.....	103.5 *	58.2 *	2.56	4.30*
Molybdenum.....	81.3 *	124.8 *	2.65	2.04
Zinc.....	11.92*	9.72*	4.02*	3.35

* Highly significant, satisfying requirements for $P=0.01$.

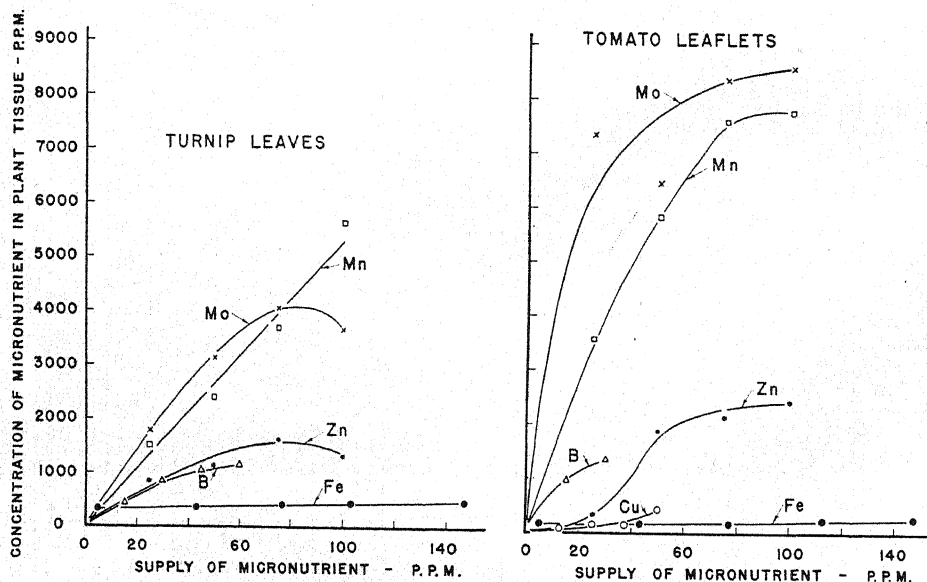


FIG. 4.—Effect of supply, in toxic concentrations, of micro-nutrients on their concentration in turnip leaves and tomato leaflets.

turnip greens are presented in table 4. Perhaps the most striking differences in vitamin content were achieved by altering the boron and manganese content of the nutrient medium. A 39% increase in the thiamine content of turnip greens resulted from increasing the boron concentration in the nutrient medium to 15.5 p.p.m. A further increase in the boron concentration in the medium was without significant effect on thiamine content. The niacin content was significantly increased, while the ascorbic acid content decreased with increments of boron in the nutrient medium. The highest niacin content resulted from the use of 45.5 or 60.5 p.p.m. of boron in the nutrient supply.

As the manganese concentration in the nutrient supply was increased, the riboflavin content of turnip greens increased, while the ascorbic acid content decreased significantly. Also, 100.5 p.p.m. of manganese in the nutrient supply resulted in a significant increase in niacin content. However, the treatment with 75.5 p.p.m. of manganese had no significant effect on the niacin content of greens when compared with the control treatment.

Several other consistent and significant trends in vitamin content can also be associated with treatment effects. Increased concentrations of molybdenum in the nutrient medium resulted in increased thiamine and niacin content of turnip greens. The highest concentrations of zinc employed were associated with the maximum riboflavin values in the experiment. The ascorbic acid content was less with increased copper supply.

Some other significant differences in vitamin content should be noted, although no consistent trends are obvious. The effects of iron, molybdenum, and copper supplies on riboflavin content of

turnip greens are of interest. In these instances a unimodal curve is obtained by plotting vitamin content against nutrient supply. The association of the two variables is certainly obscure, yet significant differences in the vitamin content of greens resulted from treatment.

The results of the analyses of variance for the tomato experiment are presented in table 5. Treatments with variable supplies of boron, copper, manganese, and molybdenum were associated with statistically significant differences in ascorbic acid content of tomatoes. In some few instances valid differences exist in the data which are primarily attributable to the influence of environment as measured by replication or by fruit-number differences.

The ascorbic acid content of tomatoes in various treatments is given in table 6. An increased supply of boron, manganese, or molybdenum in the nutrient medium resulted in tomato fruits with lower ascorbic acid values. As the copper concentration in the nutrient medium increased, however, tomatoes with significantly higher ascorbic acid content resulted. Differences as high as 65% in ascorbic acid content are demonstrable between copper treatments.

Although statistically significant differences in ascorbic acid content are demonstrable between blocks, the magnitude of these differences is considerably less (table 7) than those associated with treatment. In no case do the differences associated with replicates exceed 16%.

Discussion

It should be emphasized that plant growth in these experiments was considerably affected by the treatments employed, with the exception of the iron series. The toxic treatments of copper, zinc, molybdenum, boron, and manga-

TABLE 4
VITAMIN CONTENT OF TURNIP GREENS GROWN IN VARIOUS TREATMENTS

TREATMENT (P.P.M.)	THIAMINE		NIACIN		RIBOFLAVIN		ASCORBIC ACID	
	γ/gm. dry wt.	No. of analyses	γ/gm. dry wt.	No. of analyses	γ/gm. fresh wt.	No. of analyses	Mg. / 100 gm. fresh wt.	No. of analyses
Boron								
0.5.....	5.84	60	87.1	9	4.36	20	225.3	10
15.5.....	8.13	51	112.8	9	4.37	18	201.2	9
30.5.....	9.41	56	131.1	8	4.15	18	206.4	10
45.5.....	8.94	59	149.4	10	3.94	20	177.2	10
60.5.....	9.78	50	142.3	8	4.08	18	177.3	9
L.S.D.*.....	(1.159)		(22.04)		(0.600)		(34.99)	
Manganese								
0.5.....	6.10	57	88.4	10	3.71	20	212.2	10
25.5.....	6.09	59	95.1	10	4.16	19	198.2	10
50.5.....	5.53	57	92.1	9	4.26	18	176.9	9
75.5.....	5.73	60	95.2	9	4.49	18	180.2	10
100.5.....	5.96	59	123.0	9	5.01	20	157.1	10
L.S.D.*.....	(0.807)		(14.63)		(0.563)		(29.29)	
Molybdenum								
0.05.....	6.18	60	90.6	9	3.90	20	216.1	10
25.05.....	6.31	55	85.5	9	4.06	20	211.8	10
50.05.....	6.51	50	97.0	8	5.01	18	229.0	10
75.05.....	7.65	56	119.6	7	4.66	20	216.3	10
100.05.....	7.68	66	118.3	10	4.37	20	203.6	10
L.S.D.*.....	(1.112)		(22.10)		(0.570)		(26.63)	
Zinc								
0.05.....	6.25	63	95.3	9	3.81	20	210.5	10
25.05.....	5.49	59	77.0	9	3.85	20	257.9	10
50.05.....	5.65	45	71.0	10	3.79	20	204.0	10
75.05.....	5.30	39	84.5	10	4.63	18	209.6	8
100.05.....	4.92	26	78.9	9	5.61	14	212.6	10
L.S.D.*.....	(1.476)		(21.26)		(0.490)		(49.29)	

* L.S.D.—Denotes least significant difference between treatments calculated from analysis of variance which satisfied requirements for odds of 99:1.

TABLE 4—Continued

TREATMENT (P.P.M.)	THIAMINE		NIACIN		RIBOFLAVIN		ASCORBIC ACID	
	γ/gm. dry wt.	No. of analyses	γ/gm. dry wt.	No. of analyses	γ/gm. fresh wt.	No. of analyses	Mg. / 100 gm. fresh wt.	No. of analyses
	Copper							
0.02.....	6.28	60	91.9	9	3.74	20	216.4	10
12.52.....	6.25	54	86.9	10	4.42	20	221.9	10
25.02.....	5.72	43	92.7	10	4.55	19	191.1	10
37.52.....	4.80	26	79.2	8	4.40	12	171.9	9
50.02.....	5.90	16	72.4	8	3.86	8	178.4	9
L.S.D.*.....	(1.395)		(23.99)		(0.672)		(36.39)	
	Iron							
5.0.....	6.16	63	82.1	10	4.54	20	236.7	10
43.0.....	6.11	59	86.3	10	3.55	20	218.3	8
77.0.....	6.71	59	78.0	10	3.76	20	229.0	10
113.0.....	6.83	53	99.0	9	4.16	17	208.4	9
147.0.....	6.29	46	86.5	8	4.31	17	212.4	9
L.S.D.*.....	(1.314)		(21.52)		(0.400)		(19.63)	

nese resulted in considerably less fresh- and dry-weight accumulation of both vegetative parts and root systems than was the case with control plants. Fruitfulness of tomato plants was also seriously impaired. Thus, practical application of these results is not warranted. The data, however, do indicate some trends which merit further investigation from the practical standpoint. For instance, the association of boron supply with the thiamine content of turnip greens has possibilities. Apparently, a boron concentration of 15.5 p.p.m. in the nutrient medium seriously impaired the extent and character of root systems but did not seriously affect production of dry matter in above-ground vegetative parts. Since this treatment was also associated with a considerable increase in the thiamine

TABLE 5
RESULTS OF ANALYSES OF VARIANCE FOR
ASCORBIC ACID CONTENT
OF TOMATOES

TREATMENT	F VALUE FROM ANALYSIS OF VARIANCE FOR VARIATION BETWEEN		
	Treat- ments ^a	Fruit no. ^b	Blocks ^c
Boron.....	11.97*	3.52	0.71
Copper.....	40.63*	3.31	3.54*
Iron.....	0.36	2.52	2.74
Manganese.....	3.75*	1.38	9.67*
Molybdenum.....	17.78*	3.78	7.78*
Zinc.....	1.39	26.79*	1.72

^aFour, ^bthree, and ^cfour degrees of freedom are available for indicated sources of variation; 113-140 degrees of freedom are available in different analyses for error variance.

* Highly significant; satisfying requirements for $P=0.01$.

concentration in turnip leaves, the effects of boron supply in the nutrient medium should be carefully examined with concentrations ranging from 0.5 to 15.5 p.p.m. It is possible that a smaller incre-

severe symptoms (21) invariably contained the largest quantities of the micro-nutrient element under consideration. Turnip or tomato plants containing approximately twice as much iron as

TABLE 6
ASCORBIC ACID CONTENT OF TOMATOES IN VARIOUS TREATMENTS. TREATMENT MEANS TOGETHER WITH THEIR STANDARD ERRORS ARE GIVEN

BORON			COPPER			MANGANESE			MOLYBDENUM		
Conc. supplied in nutrient (p.p.m.)	Ascorbic acid* content	No. of analyses	Conc. supplied in nutrient (p.p.m.)	Ascorbic acid* content	No. of analyses	Conc. supplied in nutrient (p.p.m.)	Ascorbic acid* content	No. of analyses	Conc. supplied in nutrient (p.p.m.)	Ascorbic acid* content	No. of analyses
0.5	28.1±0.59	40	0.02	28.8±0.55	39	0.5	27.3±0.49	40	0.05	28.2±0.55	40
15.5	27.4±1.08	40	12.52	30.9±0.69	39	25.5	26.9±0.49	40	25.05	27.4±0.54	40
30.5	26.4±0.68	40	25.02	40.0±3.75	33	50.5	24.2±0.54	37	50.05	24.1±0.98	36
45.5	24.8±1.06	37	37.52	42.8±1.49	25	75.5	21.8±0.77	31	75.05	15.8±2.14	7
60.5	21.1±0.88	33	50.02	46.4±3.62	13	100.5	17.4±1.74	11	100.05	22.8±2.29	3

* Mg. per 100 gm.

TABLE 7
VARIATION AMONG BLOCKS WITH RESPECT TO ASCORBIC ACID CONTENT OF TOMATOES

BLOCK NO.	TREATMENTS OF					
	Copper		Manganese		Molybdenum	
	Mean + error	No. of analyses	Mean + error	No. of analyses	Mean + error	No. of analyses
1.....	36.3±1.78*	30	26.1±0.96	28	28.9±0.97	23
2.....	35.3±1.37	31	26.1±0.62	33	26.0±1.18	25
3.....	36.5±1.52	33	25.3±0.73	34	24.7±1.16	27
4.....	32.3±0.98	28	23.9±0.60	33	27.5±0.90	26
5.....	38.2±2.43	27	22.2±0.98	31	23.1±0.86	25

* All analytical results are in terms of mg./100 gm. fresh weight.

ment of boron supply would be associated with a similar change in thiamine values but with no toxic growth effects. The niacin content of turnip greens as influenced by boron supply also might have practical implications.

In general, development of toxicity symptoms in these experiments (table 8) could be correlated with results of mineral analyses. Plants exhibiting the most

control plants failed, however, to develop any toxicity symptoms, and no significant differences in growth or fruitfulness were evident.

Recently the concept that manganese is concerned in the elaboration of ascorbic acid has received some support. This concept was first developed from biochemical studies of liver tissue (29, 30), although more recent experiments (3)

TABLE 8

TOXICITY SYMPTOMS ON TOMATO PLANTS, JULY 14, 1943

(Plants had received their respective nutrients for 32 days. Those plants receiving basal solution were accepted as normal in appearance)

Supply of micro-nutrients (p.p.m.)	Whole plant	Leaves	Fruiting
Boron			
15.5	Normal size and vegetative characteristics	Brown necrotic areas, particularly at periphery of leaflets of lowermost leaves; some chlorosis in vein islets at top of plant	Normal; sepal tips brown
30.5	Tops good green color	Many lower leaves abscised; lowermost leaflets brown and dead; some chlorosis in leaflets near top of plant	Fruit small and dull green; sepal tips brown
45.5	Plants smaller; tops appear normal in color and vigor	Lower five leaves dead; partial abscission of petioles; leaflets at middle of plant necrotic, particularly at periphery, and droop with petioles at abnormal angles; possibly a nastic condition	Fruit-setting normal; sepal tips brown; occurs prior to or subsequent to anthesis
60.5	Small	Lowermost leaflets dead; lower two-thirds have necrotic areas; periphery of young leaflets near top necrotic, brown, and dried up	Fruiting appears normal; fruits dull green; lack waxy characteristics
Copper			
12.52	Lower part normal, except grayish-green coloration	Top leaflets of plants chlorotic in vein islets with general yellowing of leaflet near leaf petiole; similar to iron deficiency	Normal
25.02	Tops yellow	Leaflets small; young leaves chlorotic, particularly at leaf petiole; grayish, light-green color of leaves and stems	Fruit size reduced, with yellowish color
37.52	Tops yellow	Leaflets small with lack of pigmentation, particularly near petioles of leaves; lowermost leaflets and petioles purplish in color	Fruit yellow; sepals chlorotic; fruit small
50.02	Tops white; plants much smaller	Lowermost leaflets light green with purple on backs along veins; petioles also purple; color of leaflets becomes green, then yellow at middle, and finally white at top of plant	Calyx and corolla whitish
Iron			
43.0	Normal	All leaflets have grayish-green sheen; lowermost leaves yellow; chlorosis extending to veins and veinlets	Normal
77.0	Dark green color, otherwise normal	Back of leaflets appears to be purple in veins and veinlets	Normal
113.0	Dark green color, otherwise normal	Back of leaflets appears to be purple in veins and veinlets	Normal
147.0	Normal growth; tops normal color	Brown, spotted necrosis on leaflets of lower two-thirds of plant; more uniform necrosis on periphery	Normal; spotted dark green around collar of young fruit
Manganese			
25.5	Fair vegetative vigor	Uppermost leaves chlorotic with dark green veins and veinlets; vein islets lighter	Normal
50.5	Top appears normal	Leaflets partially dead on lower part of plant; vein islets chlorotic; brown spots on veins, veinlets, petioles, and stems of lower two-thirds of plant	Normal; sepals brown
75.5	Definite toxicity; retarded growth	Lower leaves and leaflets dark green; petioles dull, grayish brown; stems occasionally black	Fewer fruit set
100.5	Plants very small	Vein islets of upper leaves chlorotic; lower leaves mostly dead and leaflets abscised; random browning of lower stem	Fruit-setting inhibited; small in size
Molybdenum			
25.05	Tops normal	Leaflets on lower two-thirds of plant golden yellow	Normal
50.05	Plant gold in color, some green in top	Lowermost leaflets abscised; leaflets have golden brown necrotic areas; petioles a golden reddish tint	Small fruit; lower portion of fruit completely yellow with scabrous, brown collar just above equatorial portion; sepal tips brown
75.05	Extreme toxicity	Leaflets on upper plant small and chlorotic; leaflets at middle of plant golden green with yellow brown necrosis, principally at periphery; stems golden green	Scabrous brown condition at equatorial portions of yellowish fruit
100.05	Extreme toxicity; golden color	Leaflets at top of plant abscised; leaflet stems and petioles golden; remaining leaflets on lower part of plant entirely brown; necrosis occurs at periphery and progresses inward	Greatly impaired; fruit of small size; lower portion brown; cuticle disintegrated; black-brown area on equatorial portion

TABLE 8—Continued

Supply of micro-nutrients (p.p.m.)	Whole plant	Leaves	Fruiting
	Zinc		
25.05....	Slightly retarded growth, light green	Stems light green	Normal except for light yellow color of fruit
50.05....	Smaller plants; light green	Leaflets light green. Lamina chlorotic on leaflets near top of plant; lowermost leaflets contained golden yellow necrotic areas, principally at periphery	Small fruit; yellow-green; sepal tips spotted brown
75.05....	Extremely chlorotic, particularly at top	All leaflets small in size; some of lower leaflets completely yellow with necrotic areas at tips	Fruit light whitish-green
100.05....	Severe effects	All leaflets extremely small; practically white at top of plant; lower leaflets dead; back of leaflet bronzed with brown veins; top part of stem white	Flowers white; calyx whitish-yellow

have failed to confirm this conclusion. HESTER (16, 17, 18) has stated that the addition of soluble manganese to soils deficient in this element resulted in an increased ascorbic acid content of tomatoes. LYON *et al.* (22, 23) and GUM *et al.* (10), however, found manganese-deficient tomatoes in controlled culture experiments to be as high in ascorbic acid values as control fruit.

In this experiment, leaflets from tomato plants supplied with 0.5 p.p.m. of manganese contained 109 ± 3.8 p.p.m. of manganese. The tomato fruits contained 27.3 ± 0.49 mg. of ascorbic acid per 100 gm. fresh weight. When tomato plants were supplied with 25.5 p.p.m. of manganese in the nutrient, the leaflets contained 3567 ± 103.7 p.p.m. of manganese and the fruits contained 26.9 ± 0.49 mg. ascorbic acid per 100 gm. fresh weight. As the manganese concentration in the nutrient medium was further increased, the plants absorbed and accumulated increased concentrations of manganese in the leaflets, and the vitamin content of the fruit was consistently and significantly less.

More recently, HARMER and SHERMAN (15), working with spinach, oats, and sudan grass, have concluded, "The application of manganese to a manganese-deficient soil significantly increased

the ascorbic acid content of plants during the periods of the year when such manganese application caused a significant plant growth response," and "manganese played an important role in the synthesis of ascorbic acid in chlorophyll-bearing tissue, since plants growing on a soil deficient in available manganese contained less total and reduced ascorbic acid."

The data from the present report do not seem to substantiate the above conclusion that manganese plays a role in the synthesis of ascorbic acid. Leaves from turnip plants supplied with 0.5 p.p.m. of manganese in the nutrient solution contained 95 ± 3.5 p.p.m. of manganese and 212.2 ± 10.98 mg. of ascorbic acid per 100 gm. fresh weight. When plants were supplied with 25.5 p.p.m. of manganese in the nutrient solution, their growth was not significantly different in any respect from those supplied with 0.5 p.p.m., yet their leaves contained 1510 ± 48.9 p.p.m. of manganese and only 198.2 ± 15.08 mg. of ascorbic acid. The two series of plants were identical in growth characteristics and ascorbic acid content within the limits of experimental error. One series contained fifteen times as much manganese as the other by analysis. In addition, leaves from turnips supplied with 100.5 p.p.m. of manganese in the nutrient contained 5670 ± 628.0

p.p.m. of manganese and 157.1 ± 14.52 mg. of ascorbic acid. The plants exhibited definite toxicity symptoms and were stunted but contained significantly less ascorbic acid. Certainly these data would indicate that manganese is not directly concerned in ascorbic acid synthesis in turnip leaves, a chlorophyll-bearing tissue.

Since ascorbic acid oxidase has been found to be a copper-protein (20, 27) in summer squash and cucumbers, the increased ascorbic acid content of tomatoes, which resulted from supplying relatively high concentrations of copper in the nutrient medium in the experiments, is of interest.

Summary

1. Experiments were designed in 1943 to measure the effects of high concentrations of manganese, boron, zinc, molybdenum, copper, or iron in the nutrient medium on the vitamin content of turnip greens and tomatoes grown in sand cultures. Growth data as well as results of selected mineral analyses are presented, and nutritive values in terms of thiamine, niacin, riboflavin, and ascorbic acid content are considered.

2. As the iron concentration in the nutrient medium was increased to 147 p.p.m., no significant effects on the growth of either turnips or tomatoes were evident. Both plants, however, absorbed and accumulated more iron than the controls as a result of treatment, but no significant differences in vitamin content were observed.

3. As the concentration of copper, manganese, boron, zinc, or molybdenum was increased in the nutrient medium, the growth of both turnips and tomatoes was significantly retarded. Definite and specific toxicity symptoms developed, and, invariably, as the supply of any of the micro-nutrient elements used was increased, a greater concentration of that element was found in the vegetative plant parts.

4. Certain significant changes in vitamin content were striking. For instance, as the boron supply to turnip plants was increased, a 60% increase in both niacin and thiamine concentration in leaves occurred. Ascorbic acid values, however, were 20% less. A 35% increase in both niacin and riboflavin values was associated with increased manganese supply. In this instance, ascorbic acid values were 25% less. In tomatoes a 60% increase in ascorbic acid content of the fruit was associated with relatively high copper concentrations in the nutrient solution. Ascorbic acid values were significantly less than in control fruits, however, as the concentration of boron, manganese, or molybdenum was increased in the nutrient.

5. Some practical as well as theoretical connotations of these results are discussed. The reports that manganese fertilization of the soil results in high ascorbic acid values are considered in relation to these results which indicate that manganese supply to the plant has little influence on ascorbic acid content.

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NUCLEI AND CYTOPLASMIC INCLUSIONS IN BASIDIA OF AMANITA

DON RITCHIE

Introduction

The karyology of spore formation has been described in dozens of Basidiomycetes since the essential outlines were worked out by WAGER (9, 10), MAIRE (3), LEVINE (2), and others. WAKAYAMA (11, 12) has made a catalogue of chromosome numbers in several Basidiomycetes, including his own observations and those of previous workers, and his list indicates that most of the plants that have been investigated showed two chromosomes. In some species, however, notably those studied by WAGER, the chromosome count is four, six, or eight. All the fifteen species investigated by MAIRE (3) had two chromosomes, and there were seven additional species, studied by others, which also had two chromosomes. The remaining eleven species investigated had four, six, or eight chromosomes. Since MAIRE saw only two chromosomes in fifteen species in twelve genera, the fixation of those fungi may have been imperfect. Certainly such tiny particles of protoplasm can be easily misinterpreted unless they are quite distinct, and they are not distinct unless the fixation is fortunate both as to time and as to clarity of the resulting image.

Further information on basidial content was presented by SASS (7), who found dark-staining bodies associated with nuclei and with sterigma-formation in the basidia of *Coprinus sterquilinus*. He figured, for instance in the case of a uninucleate basidium, a nucleus-like object close to the real nucleus and called it a *Nebenkern*. He could not find division figures of any of the bodies and obtained varied results with different fixa-

tions. SASS suggested that the "Golgi material," since it disappears after "helping" in the formation of sterigmata, may move into the spores. He emphasized the fact that he used no osmium-containing fixatives but made his preparations with several variations of mixtures of chromic acid, acetic acid, picric acid, and formaldehyde.

The work reported here is part of a series of trials of a variety of fixations on fungi, including a repetition of the methods of SASS. The extra-nuclear bodies which he figured in both drawings and photographs are so distinct that it seems strange that others interested in fungus cytology had not, and have not until now, found similar objects in the basidia of other forms. Although it was not my original intention to seek consciously any such bodies in the several species under investigation, it was nevertheless interesting to find a fairly regular distribution of them in one plant, namely the famous Caesar's mushroom, *Amanita caesarea* Fr. Since the writer has not found them in several other species whose basidia were studied with the same techniques, it appears that the bodies are not of universal occurrence. They are either limited to certain species or else are found only at special times, those times being sporadic. The remainder of this study consisted of merely a routine examination of the basidia to check the karyology, observe the meiotic divisions, obtain a chromosome count, follow the progress of spore formation, and observe such cytoplasmic inclusions as could be found, since none of this had been done for *A. caesarea*.

Material and methods

Young sporophores were collected outdoors in midsummer, brought into the laboratory, and killed as quickly as possible in a series of fixing fluids. These included: Randolph's CRAF fixer, full and half strength (1); Randolph's plus three drops of the detergent Triton X; the Zirkle-Erliki mixture (13); Zirkle-Erliki plus 0.5% quinone; 0.5% quinone; 0.5% quinone in 0.4% NaCl post-chromed with 3% H_2CrO_4 or with $\text{K}_2\text{Cr}_2\text{O}_7$; 0.5% quinone followed by 3% CuSO_4 ; Sass's Fixative II (7); chrome-formaldehyde-copper; and chrome-acetic-copper (5). The quinone series was attempted because that substance initially showed promise of "complete fixation"—that is, a combination of both the so-called "acid" and "basic" images in the same fixed cell. The results to date have been disappointing, although NEWCOMER (4) has photographed some dividing cells fixed with quinone in which both well-fixed mitochondria and chromosomes are evident.

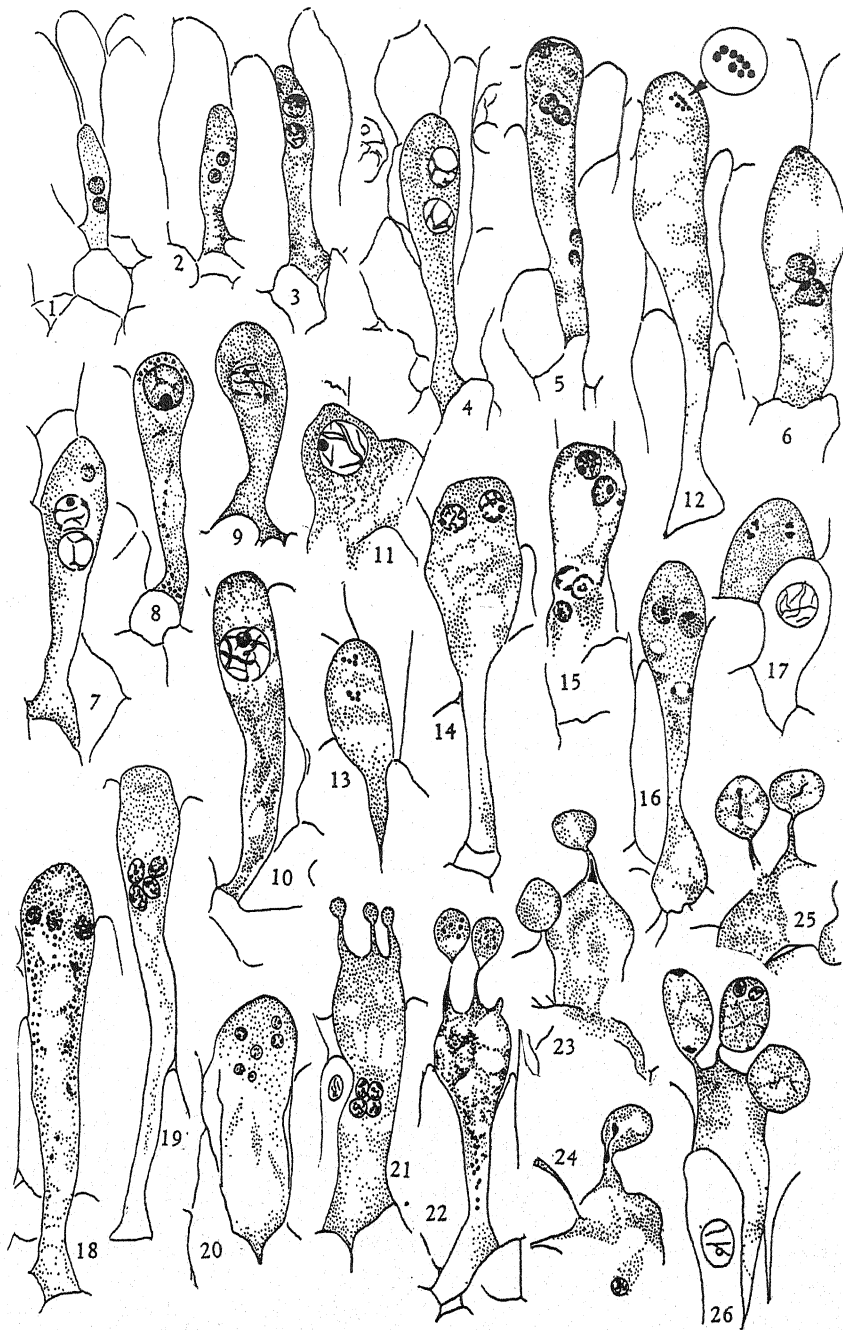
Sections of the gills of *Amanita* cut at 2 and at 4 (mostly at 4) μ were subsequently stained in iron-alum haematoxylin and with the Feulgen nucleal reaction. In the latter technique, staining was successful after 10 minutes of hy-

drolysis in normal HCl at 60° C. and 7 hours in the leuco-basic fuchsin.

Results

The behavior of the nuclei in the basidia of *A. caesarea* is typical of that of many of the Agaricaceae already studied. The cells of the mycelium below the hymenium are binucleate. The free ends of the hyphae push out through the palisade-like cellular coverings of the gills, appearing first as small, almost cylindrical cells containing two small nuclei (fig. 1). The young basidia enlarge, push out still farther (fig. 2) and eventually make their way to the surface of the gill. Meanwhile, preparatory to fusion, the nuclei enlarge to many times their original size (fig. 3). Figure 4 shows a pair of these large nuclei just before the act of fertilization. This stage resembles the second binucleate stage, but the basidium usually has not, as in this instance, grown to its full size, being still overshadowed by the surrounding larger, more mature basidia. Fusion of the two nuclei ensues (figs. 5-7). Figure 5 shows the earliest stage at which *Nebenkerne* were found, the ones in this figure being demonstrated after the same technique as that used by SASS. As SASS indicated, these bodies cannot be osmium-produced

FIGS. 1-26.—All drawn with camera lucida arm at 120 mm., mirror at 45°, paper at table level. 1640X. Fixations for indicated figures: quinone, 2; quinone plus CuSO_4 , 3; quinone plus H_2CrO_4 , 1, 8, 22; quinone plus $\text{K}_2\text{Cr}_2\text{O}_7$, 16, 18; CRAF, full strength, 4, 7, 10, 12-15, 19-21, 26; CRAF, half strength, 6; Sass's fixative II, 5; chrome-acetic-copper, 11; chromium sulfate-formaldehyde copper, 17; Zirkle-Erliki, 9, 23, 24, 25. Stains: Heidenhain's haematoxylin, 1-3, 5, 6, 8, 10, 11, 15, 16, 18, 22; Feulgen stain, 4, 7, 9, 12, 13, 14, 17, 19-21, 24-26. FIGS. 1-3, young binucleate basidia, showing general increase in size. FIG. 4, young binucleate basidium just before nuclear fusion. FIGS. 5-7, stages in nuclear fusion. FIGS. 8-11, aspects of fusion nucleus after different fixations; fig. 10 shows faintly and fig. 11 shows clearly double spireme of early prophase of first meiotic division. FIGS. 12-13, metaphase and telophase of first meiotic division, showing number, shape, and size of chromosomes. FIG. 14, second binucleate stage after first division. FIGS. 15-16, second binucleate stage similar to fig. 14, except that fig. 15 shows three extra-nuclear bodies, and fig. 16 shows two. FIG. 17, second meiotic division, telophase. FIG. 18, four-nucleate stage (only three nuclei visible in this section) with mitochondria in cytoplasm. FIG. 19, four-nucleate stage with four distinct nuclei clumped. FIG. 20, four-nucleate stage with two extra-nuclear bodies. FIG. 21, formation of sterigmata and spore initials. FIG. 22, spore initials containing mitochondria. FIGS. 23-24, migration of nuclear material into spore. FIG. 25, nuclear division in spore; see text on appearance of chromatin after Zirkle-Erliki fixation. FIG. 26, basidium with one mature, binucleate spore.



(Legend on opposite page)

artifacts. After fusion of the two nuclei, the resulting nucleus is only about forty times as voluminous as the two original basidial nuclei combined, a rather small increase as compared with that occurring in other species.

Figures 9, 23, 24, and 25 illustrate an interesting fact. The basidia from which these figures were made were killed in a "basic" fixer, the Zirkle-Erliki fluid, which is usually considered a dissolver of chromatin. Other slides, made from the same tissue and stained with Heidenhain's haematoxylin, show small, darkly staining granules which are probably mitochondria, but no chromatin appears. Such would be the usual, and the expected, image. The preparations mentioned above, however, were stained by the Feulgen method, in which chromatin material is specifically stained red or magenta. With this stain it is seen that the Zirkle-Erliki fixative does not dissolve chromatin but merely fixes it poorly and renders it unstainable by haematoxylin. Of course, with the Feulgen stain no mitochondria are evident.

The fusion nucleus then undergoes its reduction divisions. Figure 10 shows an early prophase of the first division and figure 11 a later prophase. In this nucleus the double thread of the spireme can be seen distinctly. In general, the chrome-acetic-copper fixative gives excellent images of nuclei but it is not uniform in its results and, as can be seen from this figure, is valueless for cytoplasmic detail. During the first meiotic division (fig. 12) eight chromosomes appear. Immediately thereafter the second division occurs, and in this also there are eight chromosomes (fig. 17), four going to each of the four resulting nuclei. The haploid chromosome number for this species is therefore four.

After the final division the sterigmata and spore initials appear. In this species no instances were found in which these structures appeared before the final nuclear divisions, as has been reported elsewhere (5). The nuclei move out through the exceedingly slender tips of the sterigmata (figs. 22-24) into the spores, where they divide once more. As often happens in the hymenomycetes, the mature spores each possess two nuclei (fig. 26).

The extra-nuclear bodies in these basidia deserve special note. The only reference to them in the literature of the Agaricaceae is that of SASS (7). In general, my findings are in considerable accord with his, in that the basidia of *Amanita* possess spherical objects resembling nuclei in shape and size. My figures 5 and 16—one of material fixed in Sass's fluid II and the other in quinone post-chromed—are quite like those in SASS's report, in respect to position in the cell, size, shape, and internal structure, having "an achromatic matrix containing a variable number of chromatic, crescent-shaped . . . bodies." The writer has never, however, found the extra-nuclear bodies associated with the sterigmata, nor have they always agreed in number with the nuclei. They were sometimes more numerous than the nuclei (fig. 15) and sometimes less numerous (fig. 20). Like SASS, the writer has not found them in the act of dividing. As to their origin, nature, function, and fate I have no inkling. As to their being Golgi material, I have no opinion.

The question of Golgi material in plants is so confused, and the various opinions about it are so numerous, so diverse, and so lightly supported, that it seems useless to increase the chaos by adding another cytoplasmic inclusion of unknown nature to the already long,

heterogeneous list. This much is reasonably sure: There are in some basidia certain nucleus-like bodies, fixable by ordinary cytological methods without osmium. Further, it appears that these bodies are chromatic. Although the idea of extra-nuclear chromatin has for some time been in disrepute, there has been some recent work in support of it in the motile reproductive cells of *Allomyces* (6) and in the microspores of several angiosperms (8). The Feulgen reaction, which is now generally regarded as specific for chromatin, brings out clearly stained extra-nuclear bodies in these basidia of *Amanita* (fig. 20). These are apparently the same structures which show up after haematoxylin staining (fig. 5) and which SASS figured. SASS thought that they might go into the spores, but there is no direct evidence that they do. More work, perhaps from a different angle, may explain these structures. There is always the possibility, in spite of the positive statements of some authors, that the "Golgi materials" in plants are fixation artifacts. The crucial test has never been positive; they cannot be seen in living material with present-day techniques.

Summary

1. Nuclear behavior in the basidia of *Amanita caesarea* is typical of that in most of the Agaricaceae. After fusion of the two original nuclei, the resulting large fusion nucleus undergoes two meiotic divisions, and each of the four resultant nuclei moves into a spore initial. A final division in the spore results in the formation of a two-nucleate spore. The diploid chromosome number is eight.

2. In addition to the normal nuclei, there are extra-nuclear inclusions, frequently near the base of the basidium. These inclusions resemble nuclei in shape, size, and staining reactions, being readily stained with haematoxylin after the usual fixatives, and with the Feulgen reaction. Such bodies resemble the *Nebenkerne* found in *Coprinus* by SASS.

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EFFECTS OF DIETHYLSTILBESTROL ON *ALLIUM CEPA*¹

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Introduction

Diethylstilbestrol has been reported (2, 3) to have a colchicine-like effect on mitosis when applied in very low concentrations to chick heart fibroblasts in tissue culture. LETTRÉ suggested that the benzene-C-C-N grouping found in both colchicine and diethylstilbestrol might be responsible for their similar effects on mitosis. ZOLLIKOFER (6) had earlier compared diethylstilbestrol with auxins.

In view of these findings and of the fact that in the hormone treatment of certain types of cancer there is a tendency to replace the organic estrogens by the synthetic diethylstilbestrol, it was deemed desirable to study further the effects of this synthetic hormone. Onion (*Allium cepa*) was chosen as the material for the experiments because of the presence of large chromosomes which have been observed under a large variety of experimental conditions.

Observations on this material afford comparison with the results of LETTRÉ's work on animal cells *in vitro*. Lastly, the use of this plant material provides a test of any postulated auxin-like properties of diethylstilbestrol and also serves as a source of information as to toxicity.

Material and methods

Seeds and small bulbs of *A. cepa* were used throughout these experiments. The average weight of the bulbs was 35 gm. Only bulbs with symmetrical stem bases were used.

¹ The results included in this paper formed part of a dissertation submitted in partial fulfilment of the requirements for the degree of Doctor in Philosophy in the Department of Biology at Fordham University.

The solutions of diethylstilbestrol (Merck & Co.) were prepared from stock solutions containing either 1 or 10 mg. of diethylstilbestrol per cubic centimeter of ether. All dilutions were made with ether, which was then evaporated, and 10 cc. of tap water were substituted for the evaporated ether. The suspension was then allowed to stand for 24 hours. Although diethylstilbestrol is nearly insoluble in water, several investigators have found that water-insoluble materials are able to affect the growth and development of plants immersed in suspensions. These effects have been found to be in proportion to the total amount of chemical present in such suspensions.

Three series of thirty-three seeds of *A. cepa* were germinated and grown in Petri dishes containing 10 cc. of an aqueous suspension of 10, 5, or 2.5 mg. of diethylstilbestrol. An identical number of controls was used. Bulbs with growing roots were treated with aqueous suspensions containing 100, 50, or 25 mg. of diethylstilbestrol for 1, 2, 3, 10, 24, 48, and 96 hours, with 1 week recovery in tap water. A similar group of bulbs with growing roots were treated with aqueous suspensions containing 66, 250, 500, 750, or 1000 γ of the compound for 24, 48, 72, 96, and 144 hours, with recovery of 24 and 48 hours in tap water. In all experiments with bulbs with growing roots a moist chamber was employed, and 10 cc. of suspension was used in each treatment. Controls were used for each single treatment. Excised root tips were treated with suspensions containing 10, 5, or 2.5 mg. of diethylstilbestrol for 15 minutes and for 2 hours; or with a sus-

pension containing 100 mg. of the substance for 15 and 30 minutes, and for 1, 2, and 3 hours. Controls were run in tap water for the same length of time. A minimum of five root tips of treated and control materials was used for cytological study from each single treatment.

A milligram of diethylstilbestrol was evaporated from an ether solution on the central area of 60-mm. filter paper; this filter paper was then applied to the freshly scraped stem base of a bulb. Controls were run with the untreated filter paper. After a 24-hour period of treatment the bulbs were thoroughly rinsed in running tap water and placed in fresh tap water. After a recovery period of 14 days, pieces from such treated stem bases were cut and applied for 2 days or more to freshly scraped untreated bulbs to determine whether the effects of the previous treatment could be transferred.

The observations consisted of macroscopic and microscopic examination of changes in stem and root tissue. Cytological observations were made of smears of treated and control tissues stained by the Feulgen reaction and light green.

Observations

Evidence for a colchicine-like action was not found for diethylstilbestrol within the limits of the above experiments. There was, however, a consistent auxin-like response. Changes in nuclei, attributable to toxic effects, occurred almost universally in the treated material.

Overgrowths, similar to those obtained by the use of *Bacterium tumefaciens* on *A. cepa* (1), were formed when 1 mg. of diethylstilbestrol on filter paper was applied to bulbs for 24 hours. These overgrowths reached their maximum size in 17 days. The plants, however, remained quite healthy for months there-

after and showed no signs of such tissue deterioration as was observed in bulbs infected with *B. tumefaciens*. An asymmetrical widening of the stem was observed in bulbs immersed for 2 days in suspensions containing as little as 1 mg. of diethylstilbestrol in 10 cc. of tap water.

Pieces of typical overgrowth tissue induced by 1 mg. of diethylstilbestrol were applied to healthy plants. Tests for bacteria in the overgrowth tissue were constantly negative. Two successive transfers of the overgrowth tissue resulted in the production of new overgrowths on healthy plants within 9-11 days from the time of application. Apparently a very small amount of diethylstilbestrol, if applied in very close proximity to plant tissue, can induce what may be called a localized injury response. This is in accord with the known toxicity of diethylstilbestrol. It should, however, be noted that dried sterile overgrowths did not induce this response. It may be that the living plant cells contain metabolic products which act in conjunction with the minute amount of diethylstilbestrol to induce the growth response.

The germination of seeds in suspensions of 10, 5, or 2.5 mg. of diethylstilbestrol in 10 cc. of tap water was never above 20% after 48 hours. The germination percentage of controls was 75 at that time. No increase in size of roots of the treated material occurred within the following week. Tap water (5 cc.) was added 9 days after the beginning of treatment to offset possible loss resulting from evaporation. The germination of the treated seeds on the sixteenth day of the experiment had risen to as high as 50% in the lowest concentration, while the germination of controls remained unchanged. The material was fixed and stained on the eighteenth day of treat-

ment. There was no chlorophyll present in the treated seedlings at this time, although they appeared otherwise normal, while the controls were already green and had developed adventitious roots after the usual deterioration of the radicle. Apparently the continued action of diethylstilbestrol in the above concentrations was sufficient to prevent germination of many seeds and normal development of all seeds. In most of the cells of seeds treated with 2.5 mg. of diethylstilbestrol, the Feulgen-positive material was concentrated in large nucleus-like masses with Feulgen-positive granules scattered throughout the cytoplasm. The Feulgen-positive material manifested differential stainability from bluish-pink to deep magenta. In material treated with 5 mg. of diethylstilbestrol, many nuclei were crenated. In the material treated with 10 mg. of the compound, an organized nucleus was apparently absent from some cells, and Feulgen-positive material, in the form of globules of varying sizes, filled the entire cell. These globules responded with varying intensity to the Feulgen reaction.

When seeds were immersed in the concentrations described for 24 hours only and then transferred to tap water and grown for 10 days, they showed no apparent toxic effects other than a slight reduction in percentage of germination. Cytological examination of tissues from all three concentrations revealed many pycnotic nuclei. An occasional lagging chromosome and Feulgen-positive masses in addition to the nucleus were not more frequent than in untreated seedlings of *A. cepa*. Apparently, the arrested growth and other associated toxic effects are not manifested in seedlings until after at least 24 hours of treatment with diethylstilbestrol. It does appear, however, that diethylstilbestrol inhibits

germination for varying lengths of time, depending upon the concentration used. An analysis of the selective reaction of seeds at low concentrations was not attempted.

The metaphase figures from root tip cells growing in suspensions of 1 mg. and lower of diethylstilbestrol were apparently identical with those of normal material. The chromosomes were definitely not contracted. Counts of metaphase figures made from low-power fields revealed no significant variation between treated and control material. The same conclusions can be stated for root tips grown in a suspension containing 66 γ of diethylstilbestrol.

The treatment of excised root tips in suspensions containing 10, 5, or 2.5 mg. of diethylstilbestrol for 15 minutes and for 2 hours and with 100 mg. for 1, 2, and 3 hours did not reveal a colchicine-like effect on mitosis. A 2-hour treatment with a 0.1% solution of colchicine in similar fashion produced many blocked metaphases and extensive contraction of chromosomes. A similar effect was obtained with 60 mg. of colchicine in water.

Bulbs with growing roots were placed in higher concentrations of diethylstilbestrol. A suspension containing 25 mg. of the compound was used as a treatment, and roots were removed at intervals of 1, 2, 3, 10, 24, 48, and 96 hours. The roots were fixed and stained. The entire bulb was then transferred to ordinary tap water and allowed to recover for 24 hours. The same procedures were followed with suspensions containing 50 or 100 mg. of diethylstilbestrol. The most characteristic feature resulting from all these treatments, ranging from 1 to 10 hours, was the presence of frequent anaphase and telophase chromatin bridges accompanied by breakdown of

resting nuclei into numerous granules of chromatin material. Treatment for 48 hours or longer resulted in the destruction of a large number of cells and extensive fragmentation of chromatin material. The chromatin material was observed in the form of globules of various sizes scattered throughout the cells. It was apparent that all these treatments were extremely toxic. The range of colchicine-like action for diethylstilbestrol was not revealed. If it did exist, it must occupy an extremely small range within the concentrations studied.

Discussion

ZOLLIKOFER (6) attributed auxin-like properties to diethylstilbestrol. The present work on *A. cepa* confirms this in some degree, inasmuch as a growth response was obtained which consisted of a horizontal spreading of the stem base in the bulbs to which diethylstilbestrol had been directly applied. It is possible that diethylstilbestrol is a nonspecific activator of the cell.

The data given in the present work indicate that a concentration of diethylstilbestrol lower than 25 mg. in 10 cc. of tap water has very little effect on the tissues of *A. cepa*. This may be related to the very low solubility of this compound in water. Degenerative changes induced by higher amounts of diethylstilbestrol in suspensions are very pronounced but do not differ cytologically from the effects of numerous other substances of which the toxicity for plant cells has long been established.

As regards the suggested colchicine-like action of diethylstilbestrol, no confirmation was obtained for such action on *A. cepa* in concentrations of 40–100 γ /cc. as was found by LETTRÉ (2, 3) in his studies on heart fibroblasts of chick tissue culture. The range of concentrations

of diethylstilbestrol used by the present author on *A. cepa* was within the range of concentrations used by LETTRÉ, although they were not prepared in the same manner. In addition, both higher and lower concentrations were used in an effort to find any colchicine-like action. The concentrations of 250 γ to 1 mg. of diethylstilbestrol in 10 cc. of tap water had no observable effects on mitosis in *A. cepa*. Prolonged periods of treatment with the very low concentration of 66 γ in 10 cc. of tap water gave no blocking of metaphase. The higher ranges of 2.5, 5, and 10 mg. showed neither colchicine-like action nor toxic effects within periods of time in which compounds with colchicine-like action usually have pronounced effects on plant tissue. The effects of such higher concentrations over longer periods could only be considered as toxic. It is possible that period of treatment, extremely small range of concentration of diethylstilbestrol of effective C-mitotic action, and differences of plant and animal cells must all be considered to explain differences in the action of diethylstilbestrol on animal and plant tissue such as *A. cepa*.

The results presented here have been previously reported in abstract (1). A wider publication of them seems desirable in view of the work of LUDFORD and DMOCHOWSKI (4) on the effect of diethylstilbestrol on tumors of mice. These authors reported that in the material treated with stilbestrol there occurred very few mitoses, a greater proportion than usual of metaphases, and no indication of a specific mitotic poisoning of the type induced by colchicine. In particular they reported that diethylstilbestrol did not inhibit tumor growth in the absence of toxic effects. This same conclusion was reached by the present author

in work on *A. cepa*. LUDFORD and DMOCHOWSKI stated that there is no justification for including diethylstilbestrol in the same class of mitotic poisons as colchicine and sodium cacodylate, both of which have pronounced action on the mitotic spindle. They further suggest that what was considered by LETTRÉ as colchicine-like action might have been "resting" nuclei which rounded off owing to the toxicity of the drug, simulating in this manner contracted metaphase plates. They propose to repeat the work of LETTRÉ on chick embryo fibroblasts *in vitro* and to study the results from a cytological viewpoint. It may be noted that MEIER and SCHÄR (5) have made an extensive review of the work of LETTRÉ.

Summary

Observations are reported on seeds, bulbs, and roots of onion which had been treated with concentrations of diethylstilbestrol ranging from 66 γ to 100 mg. in 10 cc. of tap water. Contrary to findings of LETTRÉ (2, 3) on animal tissue *in vitro*, no confirmation of a colchicine-like action on mitosis was obtained in the absence of toxic effects. This is in agreement with the work of LUDFORD and DMOCHOWSKI (4) on mouse tumors. A nonspecific auxin-like response was obtained on the bulbs in the form of an overgrowth of the bulb base, similar to overgrowths on bulbs infected with *Bacterium tumefaciens* (1).

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ABNORMAL PROTHALLIA IN *TSUGA CANADENSIS*

CLARENCE STERLING

Introduction

Investigations of morphological development in a plant often reveal deviations from its usual behavior. These variations may be interpreted as normal or abnormal, depending upon their frequency in the individual species or in related species. Regardless of interpretation, the record of such variations serves to indicate potentialities of behavior which lie within the range of development of the plant tissues. The investigation here reported treats of abnormalities found in mature megaprothallia of *Tsuga canadensis* Carr.

Material and methods

In the present study ovules of the hemlock were dissected for an embryological investigation. Cones were collected from a large, vigorous tree in Urbana, Illinois, between July 4 and July 17, 1947, the dissected prothallia being immediately killed and fixed in a variety of killing fluids. Sections cut at $15\ \mu$ were stained with safranin and fast green. Drawings were made by camera lucida.

Investigation

Unfortunately for the primary purpose of the study, none of the archegonia seemed to have been fertilized. This may have resulted from poor pollination in the 1947 season. Although the gametophytes appeared normal and turgid at maturity, all began to collapse and disintegrate within less than 2 weeks following the formation of the ventral canal cell.

During the 2-week period of collection, 1877 ovules were dissected. Although most of them had but a single functional

prothallium, nineteen ovules (about 1% of the total) were found with two fully developed megagametophytes (figs. 1-4). A majority of the paired prothallia were partially superposed (figs. 1-3). Four occurred with the relationship shown in figure 1; twelve were situated in the approximate position of figure 2; and two were seen as depicted in figure 3. Only one pair (fig. 4) was found in which the prothallia were virtually juxtaposed. All the supernumerary prothallia possessed a normal complement of from two to five archegonia.

An interesting feature of these paired megagametophytes is that in all nineteen pairs both prothallia were surrounded by the megaspore membrane. Thus, the contact surface between the adjacent structures was composed only of cell-wall material. The megaspore membrane was found on the outside surface of the complex, being thicker at the chalazal end and tapering out above, as also reported by THOMSON (28). Although a paired group had a larger volume than a single normal prothallium, the same peripheral form was exhibited in both cases.

Two other ovules were also discovered to show an abnormal development of the megagametophyte. In one of them (fig. 5) the archegonia were located in a lateral position (only four of the five archegonia are present in the section shown). In the other prothallium (fig. 6) two of the three archegonia were superposed, with the lower of the two lacking a distinct neck.

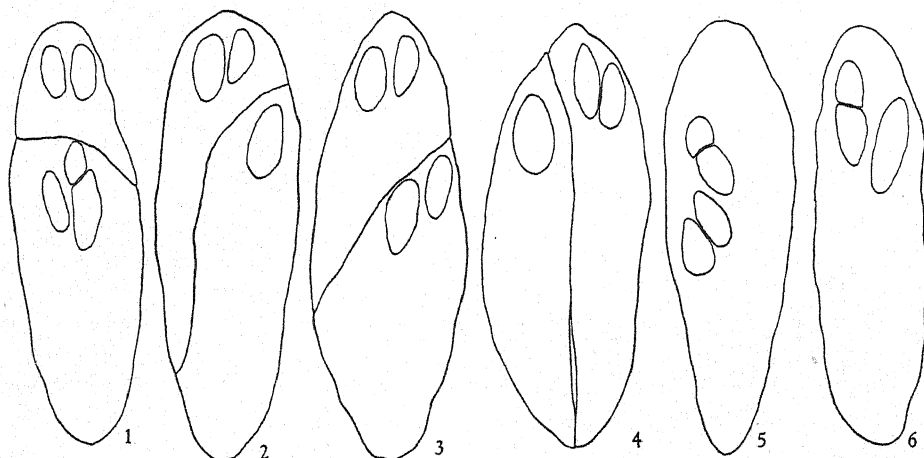
Discussion

The development of multiple megagametophytes in conifers has been mentioned frequently in morphological

studies. The question, whether the production of supernumerary prothallia in an ovule is a teratological occurrence, cannot be arbitrarily resolved. Some species are characterized by the usual germination of several megaspores in an ovule: *Sequoia sempervirens* is a notable example of this phenomenon (13, 14, 24, 28, and others). Likewise, *Austrotaxus* was described by SAXTON (23) as exhibiting a marked tendency for the develop-

above, *Taxus* has been described frequently as showing the development of at least two megagametophytes (6, 10, 11, 25, 26), and DUPLER (6) has counted as many as four and five prothallia in a single ovule. Two prothallia have been found occasionally in the nucellus of *Torreya* (19) and in that of *Amentotaxus* (27).

In the Pinaceae and Cupressaceae double prothallia seem to occur only in-



FIGS. 1-6.—Figs. 1-4. Outline drawings of longitudinal sections through double prothallia. Fig. 5, median longitudinal section of prothallium with lateral archegonia. Fig. 6, median longitudinal section of prothallium showing two superposed archegonia. All $\times 27$.

ment of more than one prothallium in an ovule. On the other hand, usually but a single megagametophyte is produced in most conifers, with supernumerary prothallia formed only rarely.

Multiple prothallia appear to occur more frequently in the genera of some coniferous families than in others. In the Taxodiaceae several embryo sacs are normal in *Sequoia* (see above) and have been noted to occur occasionally in *Taxodium* (4), *Sciadopitys* (1), and *Cunninghamia* (1). ARNOLDI (1) figured the development of five prothallia in the ovule of *Cunninghamia*. In the Taxaceae, in addition to *Austrotaxus* as described

frequently. Occasionally two prothallia have been reported in the much-investigated *Pinus* (2, 3, 7, 10, 15, 16). HAYDON (9) mentioned that he once saw five prothallia in the nucellus of *P. sylvestris*. Although some other genera of conifers (namely, in the Cupressaceae and Podocarpaceae) have likewise been found with occasional supernumerary prothallia, their recounting is unnecessary at this point. It is interesting to note, however, that multiple prothallia have not been reported in any other pinaceous genera.

The occurrence of lateral archegonia in a gametophyte may be variously inter-

preted. In the Cupressaceae such groups, which occur not infrequently in *Tetradclinis* (12, 18, 22), *Fitzroya* (5), and *Juniperus* (10, 18), may connote a near relationship to the callitroids. In families in which archegonia are normally apical (i.e., micropylar) in position, however, the formation of lateral archegonia may not be so much a phylogenetic expression as a response to locally altered physiological conditions. Among these latter might be included the lateral archegonia found occasionally in *Taxus* (25), *Torreya* (20), and *Tsuga* (17 and the present note). Lateral archegonia have also been noted in *Pinus* (3, 8, 21), and even chalazal archegonia have been described in this genus (2, 3, 16).

A question of some significance arises in connection with the position of the so-called "megaspore membrane" in those ovules of *Tsuga* with double prothallia.

The fact that such a membrane surrounds a group of two rather than each separate prothallium indicates either that the two prothallia were derived from a single megaspore (an improbable situation) or that the membrane about these double prothallia is not a *spore* membrane. With regard to the first alternative, although two prothallia ("antheridia") have been described within the microspore membrane on several occasions, such a variation in the free nuclear activity of the developing megagametophyte is notably unrecorded. If the second alternative is correct, without speculating on the morphological nature of this membrane it could be argued that, if it is not logically a spore membrane in these abnormalities, its spore nature in normal cases is suspect.

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CURRENT LITERATURE

Genera Filicum: The Genera of Ferns. By EDWIN BINGHAM COPELAND. "Annales Cryptogamici et Phytopathologici," Vol. 5. Waltham, Mass.: Chronica Botanica Co.; New York: Stechert-Hafner, Inc., 1947. Pp. xvi+247+2 figs.+10 pls. \$6.00.

Two far-reaching goals are achieved by this volume. First, all known genera of leafy ferns, including those segregated in recent years, are now assembled in a form readily available to all students of ferns and to all systematists. Older monographs and indices, such as DIELS's volume on ferns in the *Natürliche Pflanzenfamilien* of ENGLER and PRANTL (1898) and CHRISTENSEN's *Index Filicum*, with its three supplements (1906-34), are more of historical than practical importance today. CHRISTENSEN's chapter in VERDOORN's *Manual of Pteridology* (1938) is a useful summary but does not evaluate the genera completely.

Second, this exposition of modern trends in the classification of ferns is certain to stimulate thought and discussion among the current generations of botanists. It is fascinating to explore the disposition of families and genera from Pteridaceae to Vittariaceae.

Based upon the premise that pteridology has regained its position of being the best-developed field of systematic botany, COPELAND proceeds to integrate all useful knowledge concerned with the genera of ferns. Since the author has spent much of his life in the Eastern tropics and has studied all but six of the genera recognized in this work, he is in a strategic position to analyze and to synthesize the fern flora of the entire world.

The Introduction is rich historically and interpretively. The polyphyletic nature of the group known as the Polypodiaceae is stressed, and some reasons are given for the scope and sequence of the newly established families. A case is made for the return of such genera as *Phyllitis*, *Glossopteris*, *Diplora*, and *Boniniella* to *Asplenium*, while *Antigramma* of Brazil and *Schaffneria* of Mexico are kept generically distinct, largely on the basis of convenience. COPELAND treats the genus as a concrete entity and a component of nature. His definition is simple: "A genus is a single isolated species or a convenient group of related species." Emphasis is on "related," while convenience is of secondary consideration. COPELAND's studies in the Hymenophyllaceae, in the genus *Grammitis* with a hundred and fifty species, and in many other fern groups have convinced him that nine-tenths of the species and genera of ferns are Antarctic in origin.

Information in the body of the book is presented conventionally, beginning with the division of the class Filicineae into the orders Ophioglossales, Ma-

rattiales, and Filicales with one, one, and nineteen families, and four, six, and two hundred and ninety-eight genera, respectively. COPELAND is author of thirty-three of these genera and has revived or validated sixty-nine others. Analytic keys to the families of the Filicales and to genera wherever demanded are carefully constructed. Only one major error is noted—the omission of the genus *Paraleptochilus* from the key on pages 175-177. Synonyms for each genus are listed. Whether the fern is terrestrial or epiphytic in its mode of existence is specified in the description of the genus, followed by the citation of the type species. The number of species under each genus and their geographical distribution are indicated. The type of stele is used extensively in ascertaining affinities. Historical and critical remarks concerning the nomenclatural problems are to the point and generally convincing. Many readers, however, will be surprised to know the marsh fern as *Lastrea Thelypteris* (L.) Bory, the ostrich fern as *Matteuccia Struthiopteris* (L.) Todaro ("Pterelis was never tolerably published"), and the hart's-tongue fern as *Asplenium Scolopendrium* L.

Few changes have been made in the rearrangement of genera within families other than in the Hymenophyllaceae and in the Polypodiaceae as construed in the recent past. The geographic distribution of the thirty-four genera of the Hymenophyllaceae is shown in tabular form. The heterosporous leptosporangiate ferns are placed last as families Marsileaceae and Salviniaceae under the heading "Hydropterides." The latter has no counterpart in the monograph.

Of special interest is the distribution of genera in the segregates of the old Polypodiaceae. Ten families replacing the Polypodiaceae are arranged together with Parkeriaceae, Matoniaceae, and others into a unified sequence. The Pteridaceae encompasses sixty-three genera, including *Dicksonia*, *Cibotium*, *Dennstaedtia*, *Pteridium*, *Pteris*, *Pellaea*, *Acrostichum*, and *Adiantum*. The Parkeriaceae, Hymenophyllaceae, and Plagiogyriaceae are each represented with a single genus. Preceding the last-named family is the Davalliaceae, composed of three natural groups—the davallioid ferns with eight genera, *Oleandra*, and the three genera of the Boston-fern type. In commenting on the first of these groups, the author lists seven common characteristics which indicate its coherence. Similar summaries are found throughout the book. Seven genera of tree ferns are placed in the Cyatheaceae. COPELAND recognizes that a description of the largest family, Aspidiaceae, would be of little value in placing an unknown fern into its proper category, and the diversity of this group of genera is indicated by such ferns as *Onoclea*, *Polystichum*, *Athyrium*, *Cystopteris*, and *Woodsia*.

CHRISTENSEN's third supplement is followed in interpreting *Tectaria* and *Aspidium* as perfect synonyms with the former antecedent. The almost incredible variety of ways in which *Aspidium* has been construed is alluded to but not discussed in detail. *Lastrea* is considered to have five hundred species or more. A chart partly summarizes the Aspidiaceae, showing that *Rumohra*, *Dryopteris*, *Ctenitis*, *Lastrea*, and a few derived genera are organized from the *Dryopteris* of the *Index Filicum* and that three other large genera, *Polystichum*, *Tectaria*, and *Athyrium* are added. This complex of seven "old" genera accounts for fifty-six of the sixty-six genera in this family. With the increased number of genera, each has fewer species.

The five remaining families have the indicated number of genera: Blechnaceae, eight; Aspleniaceae, nine; Matoniaceae, two; Polypodiaceae, sixty-five; and Vittariaceae, nine. The Polypodiaceae is construed as an epiphytic assemblage with an abundance of tropical genera. *Polypodium* and *Phlebodium* are among the few genera which are familiar to land-bound botanists of the North Temperate Zone.

Discussions and some disagreements among contemporary pteridologists may tend to modify some of the fruitful labors presented in the *Genera Filicum*, but many praises will be voiced by those who are attempting to visualize the fern flora of the world as a unit in time and space.—PAUL D. VOTH.

An Introduction to the Science of Botany: Basic Botany. By FRED W. EMERSON. Philadelphia and Toronto: The Blakiston Co., 1947. Pp. xi+372. Illus. Frontispiece in color. \$4.00.

Gracing the washable cover of this new text is a desert scene in full color. Another innovation is the larger format which permits the use of two columns of print per page, thereby improving the speed and ease of reading.

The general plan of the book is logical. The production of corn in the Upper Mississippi Valley in 1925 is compared with increased current yield to emphasize the net gain in wealth resulting from the improvement of plants by hybridization. A discussion of life, cells, and cellular reproduction leads to chapters on leaves and their function and to cycles of energy and nutrients, including a simple nitrogen cycle. Chapters on the functions and structures of roots and stems are followed by unifying discussions on correlations, genetics, evolution of plants, and classification. Present-day plants are treated from *Gloeocapsa* to the grasses. The book concludes with chapters on plant and biotic communities. Units of the book can be rearranged easily to fit local needs. A list of paragraph titles is incorporated in the introduction of each chapter so that the unit may be visualized at a glance. Many of these titles are of necessity topical in nature, but "man's relation to the web of life," "the remedy for erosion," and "aimless destruction" create an urge to read.

Diagrams, line drawings, and halftones are of good quality and wisely chosen. It may be noted that the vascular system in two drawings of a stem tip in longisection is oversimplified.

The author includes many of the newer approaches to the study of plants, as the discussions of growth substances and of photoperiodicity will illustrate. Tabular presentations of pigments and other data on the algae are especially complete. In general, the activities of plants are described with such clarity that a student familiar with only a few of the basic facts of chemistry and physics can follow the discussions readily. Because of the basic nature of photosynthesis, it seems that this process could have been presented more extensively. Blue-green algae and bacteria are included in the phylum Schizophyta, and the use of the phyla Psilopsida, Sphenopsida, Lycopsida, and Pteropsida indicates that the author has adapted the systems of WALTER ZIMMERMANN and A. J. EAMES. It is gratifying to observe this trend in modern elementary textbooks.—PAUL D. VOTH.

Plants and Environment: A Textbook of Plant Autecology. By R. F. DAUBENMIRE. New York: John Wiley & Sons, Inc., 1947. Pp. xii+424+87 figs. Frontispiece. \$4.50.

The broad usage in America of the term "ecology" has been traditionally inaccurate both with respect to the etymology of the word and with the sense of its original usage in Europe. We now have a textbook of plant autecology that shows a comprehension of the distinctions between autecology and synecology and between these fields and those more strictly geographical and historical. DAUBENMIRE hews to the line in a formal manner until his last and, incidentally, poorest chapter entitled "Ecological Adaptation and Evolution," which is mainly on genecology. Here his work suffers, among other things, from space limitations and lack of correlation with the rest of the volume. The subject matter of the book as a whole, which treats of the environment per se and of the relations between the environment and plants as individuals, is divided into seven chapters on subjects such as the soil factor, the atmospheric factor, the biotic factor, etc. Within these groupings the material is well organized. It is simply and logically presented, so that the book itself should not interfere with the processes either of teaching or of learning. No small compliment! The photographic illustrations are all from the author's personal negatives and are well selected, but he should be dissatisfied with the poor quality of the halftones with which the publishers have provided his book. In fact, the design and typography of the book are no credit to the publishers, as it looks much like a 1920 high-school text. The reviewer does not believe in reviews that carp about minor typographical errors, slight omissions, and all the other matters that any reader can find in the best proofread books,

yet I cannot help but note the unique verbatim repetition in chapter ix of forty-four lines of pages 345-346 on the immediately following pages 348-349.

The author might have profited by reference to several books (not cited in the Bibliography) which treat at least partly the subject of environmental factors, such as REYNAUD-BEAUVERIE's *Le Milieu et la vie en commun des plantes* (1936), and RÜBEL's *Geobotanische Untersuchungsmethoden* (1922). His use of American literature, however, is good, and fully one-half of all cited papers have appeared within the decade preceding the completion of his manuscript.

The reviewer believes that DAUBENMIRE's most useful chapter is the one on "The Environmental Complex." Here one finds boldly expressed the idea of the variability, interdependence, and interaction of habitat factors; the variable significance for the organism of a factor status, duration, or change, depending on other conditions, including those of the organism itself. Although he admits that "the problem of measuring those physical conditions that really govern plant behavior is much more difficult than is commonly conceived," he does not quite warn his readers that ecological studies generally do not demonstrate causal factors but at best point up correlations and coincidences. His book is based on the prevailing tacit assumption (probably derived from plant physiology) that causal factors can be isolated and studied, but he is intelligent enough to be bothered by some of the assumptions usually made and to provide the cautions of chapter ix about the heterogeneity and dynamic nature of the environment, factor interaction, and the variability of plant requirements. That progress can be made in the field of autecology is amply demonstrated by scientific agriculture, but the situation in "nature in the rough" is another matter. Perhaps if DAUBENMIRE were a little more concerned about the difficulties (and some of the futilities) of much of ecological study, he would have devoted more than a page to the phytometer method and would have gone on from there to other "measurements" of the environment as a whole by organisms themselves and by communities. Perhaps he would not have written this book but would have done one in the field of synecology.

Students do not usually read prefaces. They are not assigned! Should they read DAUBENMIRE's, however, they would find that "the special advantage of restricting the material to this branch of ecology [autecology] consisted of making it possible for the student to devote his entire energy to a study of fundamentals [!] without having to sacrifice a large share of the time to the mastery of any of the several conflicting philosophies of plant sociology." One can have no legitimate complaint that this book treats in detail only of autecology, one branch of geobotany; but especially in view of the fact that the author considers autecology as a science "which cuts across many discrete fields of science" and that it "is indis-

pensable as a background for anyone who would undertake to grasp the complexities involved in the ecology of plant communities," one can deplore the implication of the first statement quoted in this paragraph. It is impossible to erect a notation of plant communities and, I believe, to understand the ecology of an individual on a basis only of a study of the ecosystem. If it were possible to analyze (even to recognize!) all the factors of the environment, it would be impossible to integrate the resultant data and arrive at a solution—the holocoenotic environment. Even then one would not know about the "harmonic environment"—that is, which factors are more important and at what rate they are effective. Nor would one know the extent to which the potential ecological amplitude of an organism is reduced in a particular situation by adversity and especially by competition. To be completely explicit, I do not hold that this book need cover the broader fields of synecology, plant sociology, etc., but it is necessary for the author to provide a better perspective for his readers, if only in the Preface and Introduction, so that they will not believe that all the fundamentals lie in autecology.

Despite my criticisms, *Plants and Environment* is a good and a useful book. It should be bought by all persons working not only in the ecological field per se but in the related less broad disciplines and in the applied fields.—STANLEY A. CAIN, *Cranbrook Institute of Science*.

Nucleic Acids and Nucleoproteins. ("Cold Spring Harbor Symposia on Quantitative Biology," Vol. XII.) Cold Spring Harbor, N.Y.: Biological Laboratory, 1947. Pp. xii+279+258 figs. \$7.00 (plus postage).

The rapid progress and wide scope of investigations dealing with the cytological and chemical aspects of nucleic acids and nucleoproteins can be seen in the amount of new material presented in this volume which follows by only a year the volume resulting from the Cambridge Symposium of the Society for Experimental Biology on Nucleic Acid. This volume is also a logical extension of the genetic aspects covered in the Ninth and Eleventh Cold Spring Harbor Symposia. The paper on "Chemical Structures of Nucleic Acids" is one of the last contributions of J. MASSON GULLAND, to whom the volume is dedicated. Spectrophotometric studies of the interaction of nucleic acids and nuclei with basic dyes by MICHAELIS give new insight to the basis of ortho- and metachromatic staining. Enzymatic studies on chemical structure are in papers by GREENSTEIN, CARTER, and CHALKLEY and by SCHMIDT, CUBILE, and THANNHAUSER. The action of X-rays is dealt with by TAYLOR, GREENSTEIN, and HOLLAENDER and by ERRERA. Studies on nucleic acids and nucleoproteins in viruses and bacteria include those by BELOZERSKY, BOIVIN, CHARGAFF, COHEN, HYDEN, KNIGHT, and WITKIN. Papers on

chromosomes and nuclei form another group by MAZIA, HAYASHI, and YUDOWITCH; MIRSKY; POL-LISTER and RIS; RIS; SCHULTZ; SERRA; and STED-MAN and STEDMAN. Metabolic aspects are represented in papers by BRACHET, DAVIDSON, SCHNEIDER, SPIEGELMAN and KAMEN, and THORELL. The discussions which are published provide, as usual, valuable additional sidelights on the problems presented.

Application of these findings in the field of cyto-chemistry would do much to improve the significance of its literature. The volume presents an up-to-date guide to the literature on the chemical structure of nucleic acids and nucleoproteins as ascertained by organic synthesis, enzymatic degradation, and complete amino-acid analysis (for viruses); physical and chemical properties of these substances with respect to spectrophotometry and action of ionizing radiations; the biosynthesis of nucleic acids and their possible role in metabolism; the relations of nucleic acid properties to nuclear and cytoplasmic morphology; and an introduction to certain aspects of directed mutation, embryogeny, and neoplasms. Evidence relating to higher plants is conspicuously absent.—WILLIAM L. DOYLE, *Department of Anatomy, University of Chicago.*

The Fungi, Vols. I and II. By FREDERICK A. WOLF and FREDERICK T. WOLF. New York: John Wiley & Sons, 1947. Pp. x+438 and xii+538. Illus. \$12.50.

The announcement of the publication of this two-volume text and reference book was received with considerable anticipation by students of this very diversified and increasingly important group of plants. Those who teach one or another aspect of mycology were particularly interested, for the texts which have been available for years are all out of date, and there has appeared in text form no integration of information accumulated during the last two decades—a period during which the fungi have proved to be exceedingly versatile both in the laboratory and in industry. The appearance of a work which would present both the classical aspects of mycology and a thorough treatment of the activities of fungi was timely.

The Fungi deals with the subject matter in two distinct sections: the first, comprising Volume I, presents a rather orthodox and conservative survey of the fungi, including the myxomycetes, with emphasis on the taxonomy, morphology, and, to a lesser extent, the phylogeny of the various groups; the second section, Volume II, is devoted in its entirety to the activities of fungi. The authors, in thus dividing the subject material, logically exemplify their conviction that at least an elementary knowledge of morphology, etc., is prerequisite to an understanding and appreciation of the very diverse activities of fungi.

Volume I opens with rather generalized accounts of the techniques of isolation and cultivation of fungi in the laboratory. This is followed by a short section

which treats of the principles and criteria of the classification of fungi and which concludes with keys to the classes, subclasses, and orders. The arrangement of subclasses and orders within the classes is in some cases a bit perplexing. One example is the treatment of the aquatic phycomycetes which, as the authors point out, comprise two apparently parallel evolutionary lines, the uniflagellate and biflagellate series. Yet the order Chytridiales as here treated contains both uni- and biflagellate families; the Lagerbidiiales are considered intermediate between the Chytridiales and the Blastocladiiales; and the Monoblepharidiales and Saprolegniales sit cheek-by-jowl as co-ordinate orders of the subclass Oomycetes. The problem of the Blastocladiiales indicates the inadequacy of the time-honored division of the aquatic Phycomycetes into the Archimycetes and Oomycetes: the highly developed mycelium would exclude it from the former, and isogamy in certain members of the orders would as surely exclude it from the latter. The present over-all arrangement, which suspends the Blastocladiiales between these two subclasses, would appear less natural than to consider the two series, mono- and biflagellates, as independent and parallel.

The greater part of Volume I is devoted to a descriptive survey of the fungi in which each order is described along with its characteristic morphology, reproductive structures, and processes. Practically every order is illustrated with more or less detailed accounts of a number of specific forms. Actually, it would seem that the purpose of the authors, to furnish a basis for a general acquaintance with the fungi, could have been better served by the use of fewer illustrative examples and a more comprehensive description of each of the selected few.

This volume offers only one major advantage over the standard texts on mycology, such as those of GYNNE-VAUGHAN and BARNES, GÄUMANN-DODGE, etc., with which it corresponds rather closely in context and general treatment of subject material: it is much more recent.

In Volume II, on the activities of fungi, the authors have performed a very desirable service to the student of mycology. Twenty-two chapters deal with a wide diversity of subjects, such as various physiological processes and biochemical activities; broad accounts of special groups of organisms, each of which, though taxonomically heterogeneous, comprises an important functional biological group—i.e., the human pathogens, mycorrhizal fungi, and soil fungi; effects of various agents, as radiation, medium reaction, temperature, etc., on the germination of spores and on the development of vegetative and fruiting structures; genetics and physiological specialization in fungi; host-parasite relations; associative effects; etc. Each chapter is provided with a bibliography of the source material. This novel treatment in a fully documented text is most welcome, and it is to be hoped that it will set the pattern for other authors in the future.

In general, the treatment of each of the subjects listed above is quite adequate and presents a comprehensive, if brief, account which, though not exhaustive, would serve as a guide into the literature for the more interested and curious reader. The present work, however, fails in one very important respect to render its maximal service to the biological sciences, in neglecting to integrate properly the expanding knowledge of the physiological and biochemical processes in fungi with the broader knowledge of general biological phenomena. Thus the recent and exceedingly significant investigations on biochemical mutants in *Neurospora*, etc., and the information on synthetic systems within the cell which such genic mutants has made possible, are summarily disposed of in a single short paragraph. Recent work on genetics in yeasts receives even briefer treatment. In both cases the implications of these studies are ignored.

On the whole, the work constitutes a valuable addition to mycological literature, and, by focusing attention as it does on the versatility of fungal behavior, it will remain useful for a long time to come.
—J. R. RAPER.

Botany: Principles and Problems. By EDMUND W. SINNOTT. 4th ed. New York and London: McGraw-Hill Book Co., 1946. Pp. xvii+726+403 figs. Frontispiece. \$4.50.

Three types of differences are evident when the previous edition is compared with the current revision. First, a co-ordinated effort has obviously been made to improve isolated portions of the book by clarifying certain statements, replacing illustrations, or by adding new data. The sections on metabolism, vitamin deficiencies in plants, and buds are all improved. Drawings of the grass flower, substitution of the embryogeny of *Portulaca* for that of *Capsella*, as well as the photographs illustrating hybrid vigor in maize, the anchoring of English ivy by its roots to a wall, and numerous other plates, enhance the value of this text. A few of the new illustrations seem to be less desirable than the former ones, such as the stem tip of *Elodea* replacing one of *Coleus* and the photomicrograph showing abscission of a leaf which replaces a labeled diagram. The section on heredity and variation as well as the chapter concerned with development and morphogenesis have profited by the addition of new pages, the latter to a marked degree.

Second, economic aspects of plants, present trends in botany, and the future of this science are stressed to a greater degree in the current edition. New photographs show a tea plantation, sugar cane in the field, drying of cinchona bark, tapping of a rubber tree, and the checking of erosion by contour plowing, to mention only a few. Summaries on the

economic value of stems, of ferns, and of other plant parts in the several plant groups are placed strategically in the various chapters.

Third, the inclusion of new chapters and the emphasis on plants currently used for fundamental research in genetics and biochemistry assure the interest of contemporary student populations. A new vegetational map of North America and a photograph of a pond being filled in by vegetation precede a new chapter on plant distribution. Even though plant formations are given scant attention, the principles of the distribution of plants in time and space are presented in a broad frame with discussions of antarctic floras, fossil floras, and the extent of Gondwanaland. Recent researches on the sex hormones of *Achlya*, the water mold, on the fruiting body of *Dictyostelium*, and on the genetic mechanism of the pink bread mold, *Neurospora*, are incorporated in this revision. "Botany and the Future" as the last chapter presents the electron microscope as a research tool, discusses plants useful in the production of essential substances such as penicillin and riboflavin, and shows photographs of the greenhouses of the Plant Industry Station of the U.S. Department of Agriculture at Beltsville, Maryland, and views of two well-known botanical gardens to exemplify institutions where botanical research is stressed.

The revision of the chapter on Bryophytes by HEMPSTEAD CASTLE is thorough, but one feels that the illustrations could convey the developmental approach of the text to a greater degree. Through all editions this text has been noted for the thought-provoking questions which accompany each chapter and for the helpful discussion on soils, a topic which is neglected in most elementary presentations. These features combined with those mentioned in this review make this edition a most acceptable one.—PAUL D. VOTH.

Cytologie végétale et cytologie générale. By PIERRE DANGEARD. Paris: Paul Lechevalier, 1947. Pp. 611+246 figs.

This book appears to be a well-done and rather complete summary of the recent developments in the fields of plant and general cytology. Although concerned principally with plants, it does include a number of pertinent topics dealing primarily with animal cytology and integrates the facts common to both quite well. The illustrations are mainly diagrammatic copies of the originals and do not show a great amount of detail, although in general they give the main points of interest. The literature list at the end of each chapter is fairly complete and up to date. The book can be recommended to those who may be interested in a general and well-balanced recent treatment of the subject of cytology.—J. M. BEAL.

PREPARATION OF COPY FOR THE BOTANICAL GAZETTE¹

Manuscript

A simple, direct statement of facts, shorn of all unnecessary words but not telegraphic in style, should be the contributor's goal for effective writing. It should be borne in mind that the paper is addressed to an audience of trained botanists, already conversant with the fundamentals of the science. Readers do not need to have general botanical terms defined or common botanical knowledge repeated. Some contributors, especially those with less experience, resort to much repetition in an effort to make their points clear, instead of presenting the material effectively in the first place. Many papers submitted to this journal would be greatly improved in quality by drastic cutting and more concise writing.

After the paper is typed and before it is submitted, it should be read and reread at intervals. This is the time to make revisions, not when the material is set up in type.

When historical data are presented, it is rarely necessary to review all the literature in detail or to include all the literature citations to be found on the subject. Quotations from other papers should be employed only when really essential; usually the reference is sufficient. If direct quotations are included, permission for their use should be obtained from the publisher of the material from which they are taken.

Before the manuscript is typed, a copy of the *BOTANICAL GAZETTE* should be consulted, and the style followed as closely as possible, especially regarding the arrangement of literature citations, tabular material, illustrations, headings, and subheadings. Information regarding the amount of illustrative material accepted may be found on the inside front cover of the journal. There is no arbitrary limit as to the length of manuscripts which may be accepted, but those showing evidence of carelessness or thoughtlessness in preparation will not be considered.

The following specific recommendations for preparing manuscripts are not arbitrary but are based upon reasonable requirements.

¹ A revision of an article originally published in *BOT. GAZ.* 91:327-331. 1931. A reprint may be secured upon request to the editorial office of the *BOTANICAL GAZETTE*.

1. Use white paper of the regular typewriting size ($8\frac{1}{2} \times 11$ inches).

2. Double- or triple-space the entire manuscript, *including* title, literature list, figure legends (the descriptive statements or titles printed below illustrations), footnotes, and quotations.

3. Supply first sheets, *not* carbon copies, typed with a new heavy black ribbon.

4. Make all margins at least $1\frac{1}{4}$ inches wide.

5. Avoid combining different-sized sheets or using half-sheets. If it is found necessary, after the paper is typed, to insert additional material, use full-sized sheets even if they contain only a few lines.

6. Number the sheets in the top right-hand corner only.

7. Realize that the printer is expected to set in type all that appears on the copy (printer's term for the manuscript) and note the following:

a) Any instructions to the editor or printer should be written in pencil, so that they may be erased, or, preferably, given on a separate unnumbered sheet.

b) The author's initials or name and the subject of the paper should not be typed on each sheet. If the pages are numbered consecutively, there is no risk of misplacement or loss.

c) No proofreading signs or comments should appear in the margins. If it is wished to strike out a word or line, use black pencil or ink. Do not use a delete sign or the word "omit" in the margin. It is not necessary to include marginal notations regarding the insertion of illustrations; the reference in the text is sufficient guide for the printer.

d) Additional or substitute words should be inserted in the text itself, not in the margins, using legible handwriting (not printed capitals).

e) If the changes are many, the page should be retyped.

8. Leave to the editor the indication of the different kinds of type to be used throughout the paper. If the article is long, employ brief topical headings and subheadings but do not underline them or use capitals.

9. Spell out numbers beginning a sentence. As a general rule, spell out the numbers from one to nine inclusive, using figures for higher numbers.

10. Treat **tabular material** as regular text, so far as page numbering is concerned, and insert it in the paper following the reference in the text. Unless it is impracticable on account of length, a table should be double-spaced, and the continuing text should begin on a fresh page. Since the setting-up of tabular material is expensive, the contributor is urged to include only essential data, simplified as much as possible.

11. Employ **footnotes** only when really essential and number them consecutively throughout the text. The footnote should immediately follow the line containing the reference and should be separated from the regular text by two lines drawn across the width of the page, thus:²

² Footnote.

12. Include a **summary** in all manuscripts except short ones. A **paragraph of acknowledgments**, if included, should follow the summary.

13. Double-space **literature citations** and arrange them flush with the margin, beginning a fresh page following the summary and paragraph of acknowledgments. Only titles actually cited in the paper should be included in the listed literature at the end of the paper; that is, a bibliography should not be included. Literature citations should be verified with the originals and should include all the information indicated in the following illustration:

2. ALLARD, R. W.; ENNIS, W. B.; DE ROSE, H. R.; and WEAVER, R. J. The action of isopropylphenylcarbamate upon plants. *BOT. GAZ.* 107: 589-596. 1946.

39. SATINA, SOPHIA, and BLAKESLEE, A. F. Studies on biochemical differences between (+) and (-) sexes in *Mucors*. *Proc. Nat. Acad. Sci.* 11:528-534. 1925.

Literature citations should be arranged according to the alphabetical sequence of the authors' names and referred to throughout the text by a serial number. If several titles by the same author are included, each should have a separate serial number, and they should be in chronological sequence.

14. Begin **legends for illustrations** on a fresh page, following the literature citations. This material must be incorporated as part of the manuscript, and *legends for text figures should not be attached to the illustrations*. Type the legends double-spaced and in paragraph form.

Galley proof.—Since it is assumed that the contributor has retained a carbon copy of his paper and will be agreeable to minor editorial

changes, the original manuscript is not returned to him with proof. This proof has already been read by the professional proofreaders employed by the printers, who check primarily the correspondence of the proof with the original manuscript. Any penciled queries found on the proof are raised by the proofreaders and should be answered by the author. Although proof has already had this reading, the contributor's own responsibility in proofreading is not casual. He should pay special attention to numerals, tabular matter, and the spelling of proper and scientific names. The *Manual of Style* of the University of Chicago Press and a number of other manuals and guides are available for consultation showing the customary way to indicate corrections in proof; such information may be found also in some of the dictionaries. Corrections should be made in the margins, with *black pencil*, and indicated as clearly as possible. Since changes in proof will be charged for if numerous, the contributor should refrain from unnecessary revisions.

Illustrations

1. **Consecutive numbering.**—Illustrations should be numbered in the order in which they are to appear in the text and, so far as possible, should be grouped in the order of their reference. This continuous numerical sequence should obtain even when both plates and text figures are included with the same paper. There should be only one figure 1, the first illustrations in each group being numbered to follow the last on the preceding group.

2. **Size of reproduction.**—The available space for text figures is 5 inches in width and, including the legend, 7½ inches in length. Illustrations for plates should be 5×7½ inches when reduced; that is, the proportion of the width to the length should be as 2 : 3.

METHODS OF REPRODUCTION

With rare exceptions, the *BOTANICAL GAZETTE* confines itself to two modes of reproduction of illustrations: zinc-etching and half-tone. Practically all illustrations are used as text figures. Prospective contributors who wish to prepare illustrations which would require the use of other forms of reproduction or of enameled stock in printing are advised that this will rarely be possible unless they are able to defray the extra cost.

3. **Zinc-etching.**—This method is used for graphs, charts, and all line drawings which do not have extremely fine lines or extremely small

dots. The process is less expensive than the half-tone method. Stout drawing paper or Bristol board which is dead white, not creamy or grayish, and jet-black undiluted India ink should be used for drawings. Pale ink or a wash or tint prevents the use of this process. The effect of a wash can be secured by varying the strength of the lines and the density and size of the stippling. Bold drawing on a scale large enough for half or more reduction will give better results than fine work done under a lens on a scale permitting only slight or no reduction. Fine dots are not only unnecessary but often disappear in reproduction. Care should be taken to maintain the same scale of strength in all the lines and the same general size in all the printing or lettering.

For graphs and charts use India ink on co-ordinate paper ruled with *light blue lines only*.

4. **Half-tone.**—This method is used for the reproduction of photographs and microphotographs. In making the negative, use a "contrasty" film or plate, develop with a "contrasty" developer, and print on glossy black-and-white paper. Contrast should be overemphasized, since some of it is lost in reproduction.

MOUNTING

5. **Trimming.**—All unnecessary background should be trimmed before the figure is mounted. Trim away excessive blank paper around the drawings and all except the object to be illustrated in photographs or photomicrographs.

Such trimming lessens the amount of reduction required.

6. **Background.**—Pure white cardboard or Bristol board should be used in mounting drawings or photographs. In all cases the background should be firm enough to support the individual figures without wrinkling. Wrinkles in a photograph tend to show as shadows in reproduction. Library paste is not permanent enough for use in pasting. Use rubber cement or photographic mounting tissue for attaching the figures.

7. **Grouping.**—Text figures should be mounted in groups whenever possible, using the full page width (5 inches). After the margins are trimmed, the figures should be mounted as closely as possible. Economy of spacing means less reduction in reproduction, a distinct advantage when fine details are to be shown as clearly as possible. Often the full width of the page can be utilized by placing two figures side by side rather than one under the other, and the result is more pleasing. Examine previous issues as guides for grouping and other matters.

8. **Index lettering.**—It is most important to use letters and figures large enough to reduce with the rest of the illustration and retain their legibility. After reduction they should be at least $\frac{1}{16}$ inch high. The smallest size used obviously should be the one considered in this connection. Index letters should be printed or mounted reasonably close to the object they designate.

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